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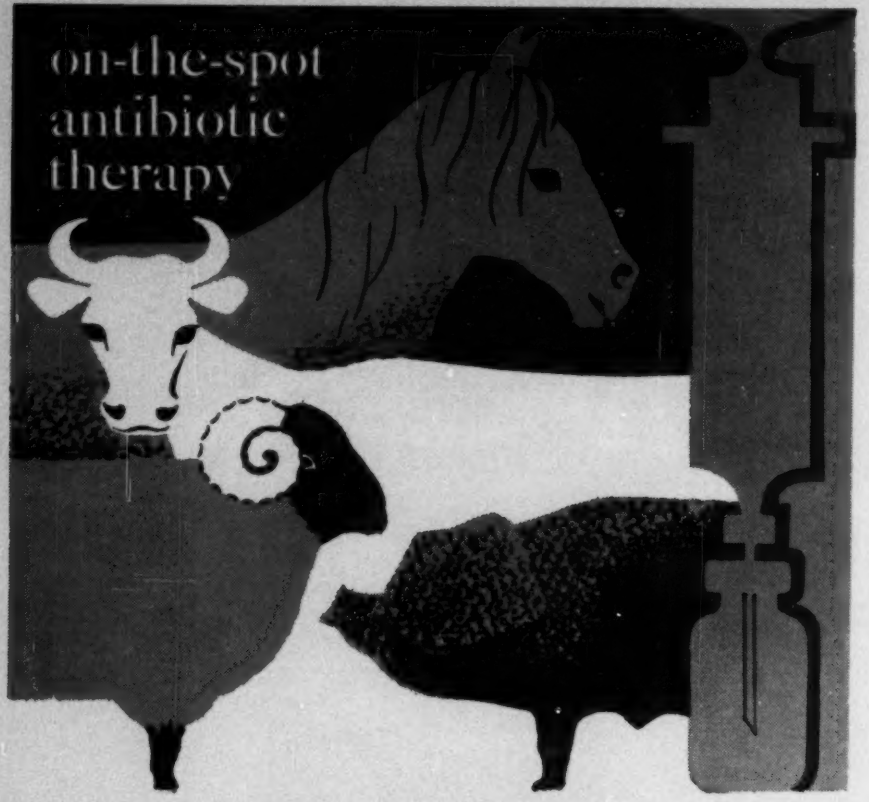

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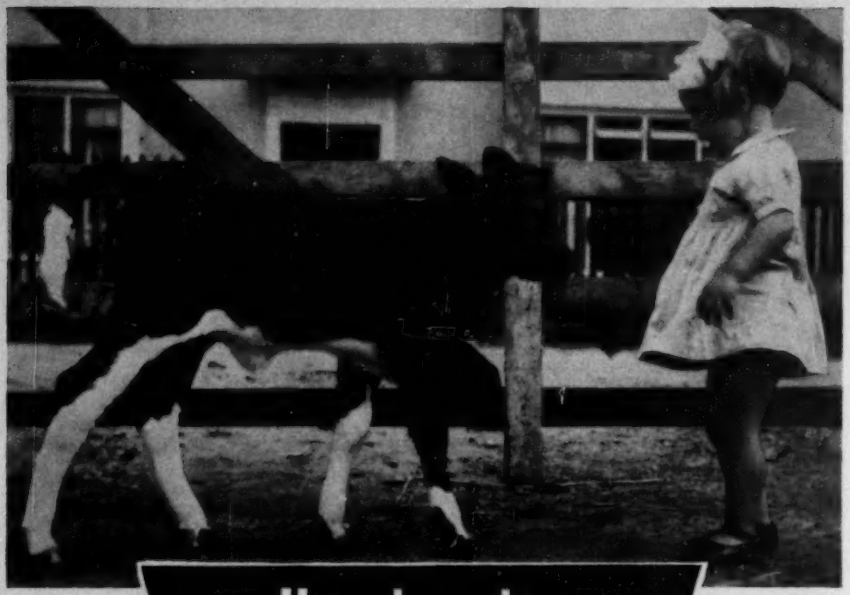

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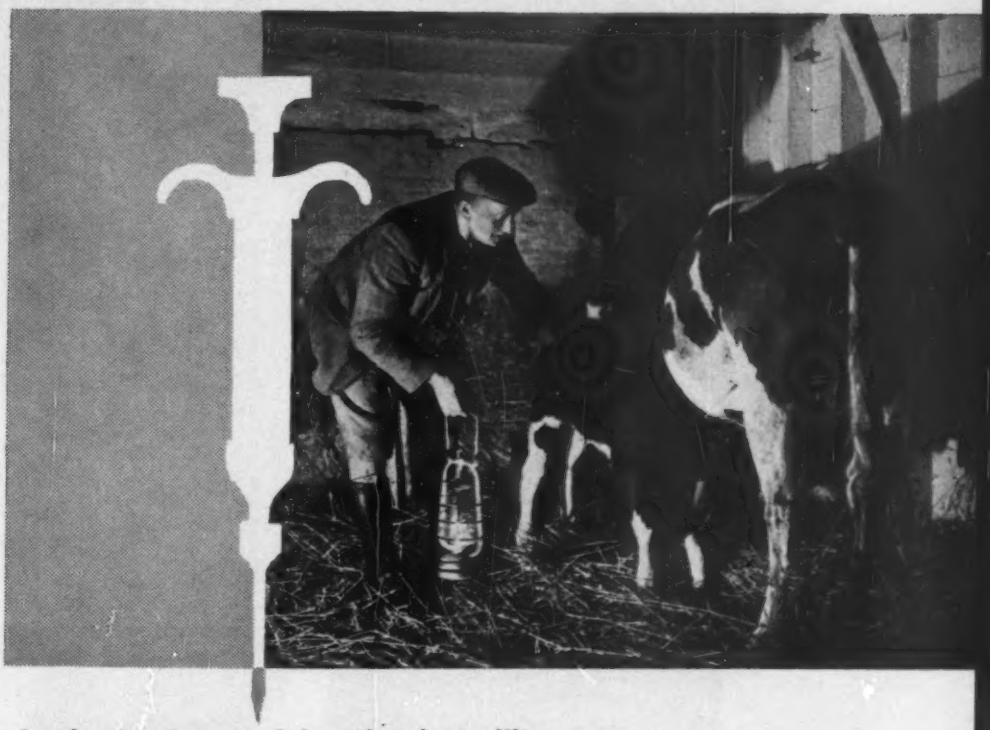

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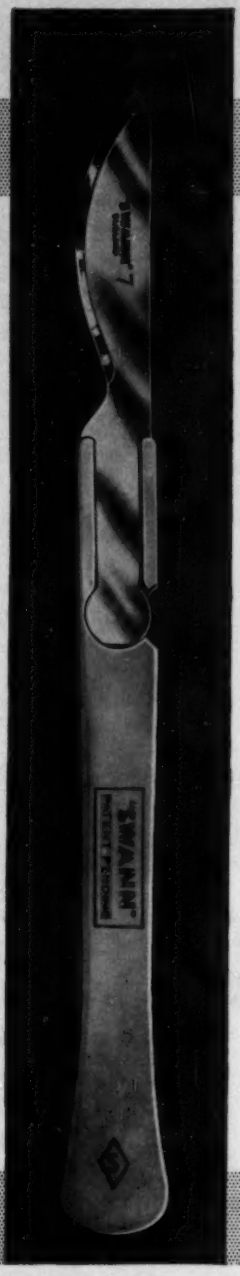

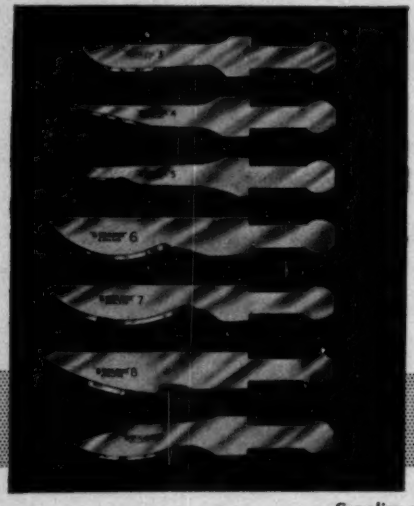

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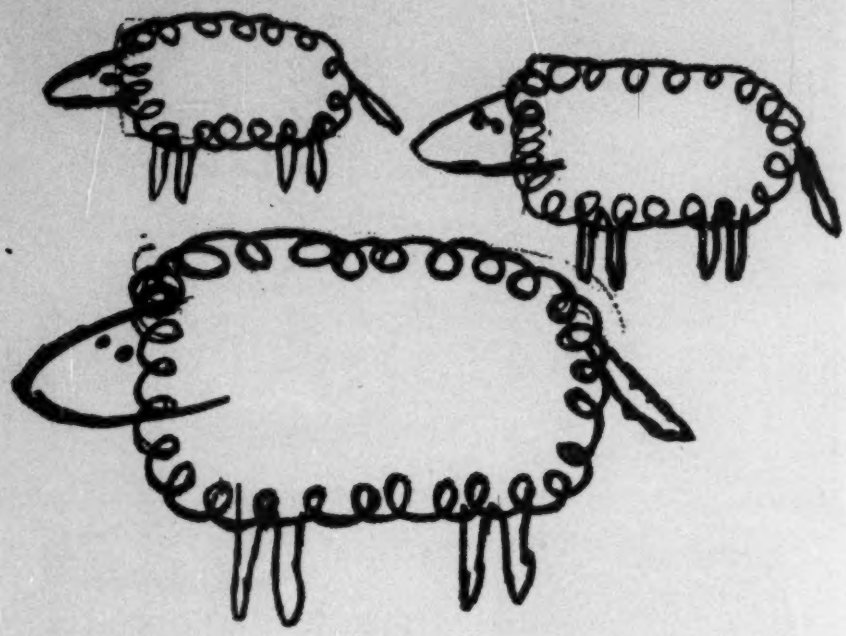

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OVINE BRUCELLOSIS:
A review of the disease in sheep manifested by epididymitis and abortion

By W. E. Lawrence
Department of Veterinary Pathology and Bacteriology, University of Sydney, Australia

HISTORY OF THE DISEASE

Epididymitis has been regarded as an important cause of infertility in rams for many years. In 1942 Gunn, Sanders and Granger drew attention to the condition after examining more than 9,000 rams in Queensland and New South Wales, when they found that approximately 5·5 per cent showed clinical abnormalities. Their description of the disease, which they called "epididymitis with spermatocoele", and its pathology indicates that the syndrome they were dealing with was almost certainly that now known as ovine brucellosis. From semen examinations they carried out these workers suspected a bacterial cause, but they could not find any bacteria in post-mortem specimens.

In the tropical north-west of Queensland, Moule (1950) found that 18 per cent of 972 rams examined showed epididymitis, but he also could not find any infective organisms from post-mortem specimens. However, the 1950–51 Annual Report of the Commonwealth Scientific and Industrial Research Organization of Australia (Anon., 1951) mentioned a condition, recognized for several years in the New England district of New South Wales, in which abortions occurred in ewes so late that the foetus appeared to be born dead at full term. Rickettsia-like micro-organisms were found in stained sections of foetal material.

In New Zealand epididymitis in rams was also causing concern (Crawford, 1949), but it was during the investigation of a flock with a high incidence of abortions that McFarlane, Salisbury, Osborne and Jebson (1952*b*) found an organism which they first thought to be similar to that causing abortions in ewes in Scotland (Stamp, McEwen, Watt and Nisbet, 1950). Thinking it to be of a rickettsial nature they cultured it in the yolk sacs of chick embryos for some time (Hartley, 1958, personal communication) before realizing that it was a coccobacillus and would grow on blood agar in an atmosphere of increased carbon dioxide. Further investigations (McFarlane, Jebson, Hartley, Salisbury, McClure and Osborne, 1952*a*) showed that the same micro-organism could be recovered from many cases of epididymitis and that both disease of the foetal membranes of ewes and epididymitis of rams could be reproduced with this agent.

In 1953 Simmons and Hall described a "brucella-like" organism which they had isolated from natural cases of epididymitis in rams in Queensland, and shortly afterwards Buddle and Boyes (1953) reported their studies on the

New Zealand bacillus which they thought might be a stabilized mutant of *Brucella melitensis*. Since then the organism, associated with epididymitis, has been reported from other parts of Australia (Gorrie, 1953; Symons, 1953).

In California, McGowan and Shultz (1956) reported an epididymitis of rams similar to the disease in Australasia; the pathology and bacteriology of the condition was described by Kennedy, Frazier and McGowan (1956).

In South Africa Van Rensburg, Van Heerden, Le Roux and Snyders (1958) found brucella-like organisms, associated with epididymitis, in semen and they showed that these organisms were probably identical to those found in Australia and New Zealand.

The bacillus has also been reported in eastern Europe (Gdovin, Hruda, Chládecký & Koppel, 1955; Gdovin, Hruda & Koppel, 1956; Tudoriu, Andrei, Drăghici & Moldoveanu, 1958).

Reports of abortions associated with *Brucella ovis* have come almost solely from New Zealand. In Australia Keogh, Doolette and Clapp (1958) recorded abortions in three flocks in which *Br. ovis* was confirmed or suspected and Simmons (personal communication, 1960) stated that abortions due to *Br. ovis* infection had occurred in one flock in Queensland.

LESIONS: (A) RAM

In the ram, lesions are generally confined to the contents of the scrotum. The most constant lesion is an enlargement of the tail of the epididymis, usually accompanied by varying degrees of adhesions between the layers of the tunica vaginalis. Sometimes only the head may be affected, at other times the whole organ. Unilateral and bilateral lesions are both seen (Gunn *et al.*, 1942; Doolette, 1953; Miller & Moule, 1954; Jebson, Hartley, McClure & McFarlane, 1955; Kennedy *et al.*, 1956).

Jebson *et al.* (1955) described two types of reaction. An acute form was sometimes seen in natural cases and more often in experimentally infected rams. This was a painful inflammation with oedema involving both testis and epididymis, although it might be confined to a localized oedema of the epididymis with fluid in the cavity of the tunica vaginalis. This acute reaction was also seen in experimental rams by Simmons and Hall (1953) and Littlejohns and Lawrence (1959, unpublished).

A chronic reaction might follow after the acute reaction had subsided or it might develop as the initial reaction to infection. The chronic reaction produced an enlarged hard tail and thickened tunics. Fibrosis might involve the tunica dartos, resulting in a reduced mobility of the testis in the scrotum (Jebson *et al.*, 1955).

The increase in size of the tail could vary from a slight enlargement to a four to five-fold increase (Kennedy *et al.*, 1956). Spermatocoeles might occur (Gunn *et al.*, 1942; Miller & Moule, 1954; Jebson *et al.*, 1955; Kennedy *et al.*, 1956) and when they did the gross lesions developed more rapidly (Miller & Moule, 1954), probably because of a tissue reaction to the extravasated sperms. The spermatocoeles might cause obstruction with a damming-back of spermatozoa in dilated tubules. Even without spermatocoeles there has been evidence

of a partial obstruction (Jebson *et al.*, 1955), this probably contributing to their formation. Involvement of the testis, which might show fibrosis and atrophy (Gunn *et al.*, 1942; Simmons & Hall, 1953; Miller & Moule 1954; Jebson *et al.*, 1955; Kennedy *et al.*, 1956), was usually associated with complete obstruction of the epididymis according to Jebson *et al.* (1955).

Histology

The histology of the lesions in natural cases has been described briefly by Jebson *et al.* (1955), and the histology of experimental infections in greater detail by Kennedy *et al.* (1956). The latter authors infected rams by injections into the testes and found that the initial lesions were always confined to the tail of the epididymis. These were essentially perivascular in distribution, with perivascular oedema and loose accumulation of lymphocytes involving not only the interstitial vascular system but also the capillaries in the basal layers of the tubular epithelium and peritubular musculature, and vessels of the tunica albuginea and muscular coat of the vas deferens. The lesions in the natural cases were essentially the same, with plasma cells present in both. Later the oedema became more diffuse, with more extensive cellular infiltration, and the tubular epithelium underwent hyperplasia with the development of intraepithelial cysts. The epithelial proliferation together with the interstitial oedema produced a narrowing or obliteration of the lumen of the tubule with proximal stasis of spermatozoa.

The inflammatory oedema was replaced by granulation tissue and fibrosis and there was also a granulomatous reaction to the spermatozoa in the obstructed tubule and in the proximal part of the tubule where damming-back of the sperms resulted in extravasation. Foreign body giant cells appeared in these areas.

LESIONS: (B) EWE

Lesions in the ewe have been well described by McFarlane *et al.* (1952*a*, *b*) in naturally infected sheep, and very briefly by Hartley, Jebson and McFarlane (1954) in experimentally infected ewes. Although the description by McFarlane and his co-workers was made before the nature of the organism was understood, there seems to be no doubt that the organism causing lesions was indeed *Brucella ovis*. Buddle and Boyes (1953) showed that the organisms obtained from infected ram genitalia and from infected foetal membranes were identical.

The placental lesions were very obvious, the most striking feature being a gross gelatinous oedema of the normally thin transparent chorio-allantois (McFarlane *et al.*, 1952*b*). Usually the membrane was increased in thickness to 2 to 3 cm., and it might exceed 5 cm. Oedema was often restricted to one horn, the tip being most affected usually. The amnion adhered to the chorio-allantois and had thickened areas, often stained with bile pigments. Changes in the cotyledons varied from a slight firmness and lightening of colour to a grossly diseased tissue, very firm, yellowish-white and very enlarged. Firm, elevated yellowish-white plaques occurred in the intercotyledonary areas, at first small but tending to coalesce into large areas.

Hartley *et al.* (1954) reported that smears from diseased foetal membranes showed brucella-like organisms either free or within both neutrophils and desquamating chorionic epithelial cells, after experimental infection by the intravenous route. In dead lambs there were usually no gross lesions from infected foetal membranes, but in two cases thickening and necrosis of the umbilical cord was seen. *Br. ovis* could be detected in a large percentage of lambs born dead, in the abomasal contents and the lungs, being more plentiful in the former and both free and within neutrophils. Cultures were obtained from these organs and the spleen. Organisms could not be found when there was no apparent infection of foetal membranes.

BACTERIOLOGY

The brucella-like nature of the causal organism was emphasized by Simmons and Hall (1953) and Buddle and Boyes (1953), although it does not conform to any of the three classical species. The latter workers thought it might prove to be a mutant or variety of *Br. melitensis* but later Buddle (1956*b*) proposed that it be given specific status with the name *Br. ovis* (new species).

The organisms are small bacilli or coccobacilli with straight axes 0·7 to 1·2 μ long by 0·5 to 0·7 μ wide occurring singly or in pairs. They are non-sporing and are Gram-negative. Like *Brucella abortus* they resist decolorization with weak acids when the modified Ziehl-Neelson stain of Stamp *et al.* (1950) is used, but in contrast to *Br. abortus* they stain blue by the modified Koester stain (Cristoffersen and Ottosen, 1941).

Br. ovis appears to be serophilic, growing well with 10 per cent serum or blood but poorly in their absence. It requires an atmosphere of 10 per cent carbon dioxide for primary isolation. Simmons and Hall (1953) obtained optimum growth by reducing the atmospheric pressure to 21 cm. Hg after replacing air with carbon dioxide and incubating at 37°C.

In 48 hours at 37° colonies of 0·5 mm. diameter are visible, increasing to 2 mm. at five days (Buddle & Boyes, 1953). In a five-day culture colonies vary in size from 0·5 to 2 mm. diameter. At 34° growth is similar to that at 37°, while at 27° the same size colonies appear in approximately twice the time (Lawrence, 1960, unpublished). Haemolysis of blood does not occur.

Colonies are circular, convex, amorphous, smooth, with an entire edge and glistening, greyish-white by reflected light; when examined by obliquely transmitted light they are uniformly of a non-smooth type (Buddle, 1956*b*). The rough nature of the bacillus is shown also by the acriflavine test of Braun and Bonestell (1947) and the thermo-agglutination test of Wilson (1933).

Buddle's studies (1956*b*) showed a lack of urease activity in contrast to the vast majority of strains of *Br. abortus*, *Brucella suis* and *Br. melitensis*. *Br. ovis* also failed to produce hydrogen sulphide. Both Simmons and Hall (1953) and Buddle and Boyes (1953) found that the organism was unable to produce indole or reduce nitrates, failed to change litmus milk or liquefy gelatin, and gave negative methyl red and Voges Proskauer tests. Kennedy *et al.* (1956) recorded a strong catalase production.

In dye-sensitivity tests *Br. ovis* did not correspond to any of the three

recognized species of *Brucella*. Like *Brucella abortus* it was very sensitive to methyl violet and grew in the presence of thionine, but unlike *Br. suis* it grew in the presence of basic fuchsin although according to Buddle (1956*b*) lower dilutions caused inhibition, while Simmons and Hall (1953) found no inhibition. Buddle (1956*b*) used the sodium diethyldithiocarbamate test of Renoux (1952) and claimed a similar result to that found with *Br. melitensis*.

Whether *Br. ovis* is a true *Brucella* or not appears to be still a matter for debate. Meyer and Cameron (1956) in metabolic studies found that, unlike smooth or rough stains of *Br. melitensis*, *Br. ovis* failed to utilize carbohydrates. However, Buddle (1956*b*), using the technique of Pickett and Nelson (1955), reported preliminary tests showing fermentation of glucose, maltose, mannose and trehalose. Meyer and Cameron claimed that it resembled the *Neisseria* species in its metabolic activity rather than the *Brucellae*. Kennedy *et al.* (1956), who emphasized the pleomorphic nature of the organism, though it shared many characteristics with the *Haemophilus* genus. Renoux (1957) did not think that there was any definite reason why the ovine organism should be considered a mutant from known species of *Brucella*. He thought then that it was possible to include it in the genus *Brucella*, the only remaining problem being how to label it.

The rough nature of *Br. ovis* results in unstable suspensions in normal saline at neutral pH, but Buddle (1956*b*) found that suspensions in buffered saline at pH 8·4 remained stable at 37° for three days and were not agglutinated by normal rabbit or guinea pig sera. Renoux and Mahaffey (1955) used 10 per cent normal rabbit serum to suspend rough strains of *Brucellae* and avoid auto-agglutination, but this was not successful with all strains of the ovine organism. Clapp (1957) obtained relative stability by suspending the organism in a dilute solution of NaOH, pH 11·0.

By the use of serum agglutination and cross-absorption tests Buddle (1956*b*) showed that strains of *Br. ovis* from New Zealand, Queensland and New South Wales, Victoria and South Australia were the same antigenically, and that they all shared antigens with rough strains of *Br. abortus* and *melitensis* but did not possess the smooth antigens A and M. Clapp (1957) had similar results with strains from South Australia, Queensland and New Zealand. Renoux and Mahaffey (1955) by using cross-absorption tests declared the existence of an antigen "r" which is common to all strains of *Brucella*, and which can exist alone in the rough forms of these bacteria: it is present in *Br. ovis*. They also found another antigen, which they called "z", present in all the *Brucellae*, but in large quantities in *Br. ovis*. Buddle's studies (1956*b*) indicated that the ovine organism possessed a common rough antigen with non-smooth *Brucellae* and also two antigenic factors which varied quantitatively in individual strains. The two factors were present in much smaller quantity in non-smooth *Brucellae*.

Vaccination with *Br. abortus* Strain 19 did not produce agglutinins against *Br. ovis* and serum from rams infected with *Br. ovis* failed to agglutinate *Br. abortus* (Clapp, 1953).

Complement fixation has been used to detect serum antibodies to *Br. ovis* and the test is now used routinely in Australia and the U.S.A. Three different

antigens have been employed. Clapp (1953, 1955) found that to overcome the strong anticomplementary activity of a simple cellular antigen it was necessary to emulsify the growth from blood agar plates in a 15 per cent guinea pig serum-saline mixture at 37° to allow the complement to be absorbed; the organisms were afterwards killed and any free complement inactivated by being heated at 60° for one hour. The antigen thus produced was not entirely free from anticomplementary activity. To compensate for this Clapp used three minimum haemolytic doses (M.H.D.) of complement; his haemolytic system contained final dilutions of 3 per cent sheep erythrocytes and five M.H.D. haemolysin.

Gorrie (1953) at first used a boiled unfiltered bacterial suspension, but for some time (1958, personal communication) he has used an antigen of washed cells extracted by autoclaving at 120° for 30 minutes and this has proved more satisfactory. Sometimes the whole cell antigen could be used, but at other times it proved anticomplementary and after centrifugation the supernatant usually made a satisfactory non-anticomplementary antigen although activity might be slightly reduced. Clapp (1958, personal communication) also found that hard centrifugation reduced the anticomplementary activity. Gorrie (1959) considered that antigens could only be relied on for three months before deterioration began.

Biberstein and McGowan (1958) subjected formolized cell suspensions to sonic vibration and used the supernatant after centrifugation, as an antigen. This removed the anticomplementary activity of the whole cell suspension. They found that storage of the antigen at very low temperatures resulted in more rapid deterioration of activity than storage at 4°.

PATHOGENICITY

Natural infection has not been recorded in animals other than sheep. In guinea pigs Buddle and Boyes (1953) at first reported progressive emaciation and death in approximately four weeks after intraperitoneal injection of 10^9 organisms, when they recovered them from lungs, liver and spleen. Later with higher doses Buddle (1956*b*) found splenic infection but no macroscopic lesions when guinea pigs were killed six weeks after inoculation. Simmons and Hall (1953) recovered the organism from one of six guinea pigs when the animals were killed eight weeks after inoculation, and they failed to infect rabbits or mice. Gregory (1955) needed 10^9 bacilli to produce a low complement fixation titre in guinea pigs but could not recover the organism six weeks after inoculation. However the Commonwealth Scientific and Industrial Research Organization's 9th Annual Report (Anon., 1957) stated that some adult guinea pigs harbour *Br. ovis* in the spleen for as long as 50 – 70 days after intraperitoneal injection, while in females infection of the foetuses may result in stillbirth or neonatal death.

Rabbits have generally shown no macroscopic lesions although Buddle (1956*b*) found necrosis of one testis and acute inflammation of the corresponding epididymis in two animals after intravenous inoculation, the organism being recovered from the tail of the epididymis and the spleen.

Kennedy *et al.* (1956) easily infected rats by intratesticular injection and the resultant disease was roughly analogous to that in rams, with epididymitis, orchitis and periorchitis. As in rams the tail of the epididymis was most commonly affected, a notable feature being the large numbers of organisms present.

TRANSMISSION

(a) *The natural disease*

In the natural disease the ram excretes *Br. ovis* in infected semen and may continue to do so for long periods. Buddle (1956*a*, *b*) said a high proportion of rams secreted infected semen for longer than three or four years and Hartley, Jebson and McFarlane (1955) found that some rams could excrete the organism for at least two years.

Infected material from the ewe can include foetal membranes, vaginal discharges and milk, besides the aborted foetus or dead lamb (Buddle, 1956*b*). Hartley *et al.* (1955) found viable organisms in vaginal discharges for at least ten days after parturition in ewes which had infected foetal membranes. Watts (1955) records *Br. ovis* in "the fluid expressed from ewes' mammary glands"; Wellington (1955) mentions cases of mastitis in ewes with *Br. ovis* in the mammary secretion and Snowden (1957) says *Br. ovis* was cultured from the milk of some cases.

Transmission from ram to ram via the ewe's vagina seems to be the most common method of spreading the infection (Hartley *et al.*, 1955; Buddle, 1955; Clapp, Keogh & Doolette, 1957; Snowden, 1958; Keogh *et al.*, 1958). Keogh *et al.* (1958) quote Richards (1957) as having observed that one ewe might be served by three rams during the one heat period in flock matings, although the frequency diminished as the mating season progressed. By this method of transmission the incubation period before lesions appeared was eight to twelve weeks in the experience of Hartley *et al.* (1955).

Infection of ewes when they are mated with infected rams seems to be much less frequent. Hartley *et al.* (1955), using microscopical and cultural examination of foetal membranes after the ensuing pregnancies, failed to find evidence of infection in ewes mated under natural conditions to infected rams. However, Buddle (1955) showed infection in two out of 25 ewes mated to five infected rams, both ewes having serum agglutinin titres, infected membranes and dead lambs. The appearance of complement-fixing antibodies in ewes after mating with infected rams, without clinical evidence of infection, was demonstrated by Clapp *et al.* (1957), Snowden (1957) and Keogh *et al.* (1958). Keogh *et al.* (1958) observed that these ewes rarely developed titres within the first few weeks of mating and titres might not appear until close to lambing. Both Keogh *et al.* (1958) and Snowden (1958) found that titres may be lost within a few months or persist for years.

Hartley *et al.* (1954) could not transmit infection from experimentally infected ewes to clean rams or vasectomized rams, and Buddle (1955) failed to demonstrate active infection of four rams running with 27 ewes which had gross infection at the last parturition, although two rams developed serum

agglutinins to *Br. ovis*. Snowden (1957) had similar results using complement-fixation-positive ewes. Infection in the ewe seems rarely to persist from one season to the next (Hartley *et al.*, 1955; Buddle, 1955).

Transmission of infection between rams without the presence of ewes is of some importance. This has been shown to occur by Buddle (1955), Gdovin *et al.* (1956), Clapp *et al.* (1957) and Snowden (1957). Snowden described homosexual practices amongst one-year-old rams although actual penetration was not observed. However, Gorrie (1957) produced both lesions of epididymitis and a positive complement fixation titre in a clean ram by rectal instillation of *Br. ovis*.

Lambs born of ewes with grossly diseased placentae failed to develop active infection (Buddle, 1955), although newly born lambs might show strong complement fixation titres. These antibodies are probably passively transferred from the dams, as they decline and disappear within a few months (Wellington, 1955; Clapp *et al.*, 1959) or weeks (Snowden, 1957), although the lambs may be drinking infected milk during this time. Snowden did not observe any infection in young rams under these conditions after 14 months.

Ewe to ewe infection when the animals are grazing together, or via contaminated pasture, could not be demonstrated by Buddle (1955). Oral infection from contaminated pasture seems to play little or no part in the natural transmission of the disease either in ewes (Buddle, 1955) or rams (Hartley *et al.*, 1955; Clapp *et al.*, 1957) despite Buddle's (1957*b*) observation that *Br. ovis* can survive in infected material on pasture for several months under favourable conditions.

(b) *Experimental transmission*

Various workers have been able to reproduce the disease in sheep by parenteral injection of *Br. ovis* organisms or by applying them to mucous membranes.

Ewes were infected by intravenous injection of *Br. ovis* by McFarlane *et al.* (1952*a*), Hartley *et al.* (1954), Jebson, Hartley and McFarlane (1954), and both orally and intravenously by Buddle (1954), during early pregnancy, resulting in infected membranes and dead lambs. Ewes which were experimentally infected shortly after mating aborted from 40 days' gestation up to full term, while others produced dead lambs or lambs which died within 24 hours of birth (Hartley *et al.*, 1954).

Rams were infected by intravenous and intratesticular injection or by instilling the organisms into the prepuce (McFarlane *et al.*, 1952*a*; Hartley *et al.*, 1954; Jebson *et al.*, 1954). Simmons and Hall (1953) could only infect rams by intratesticular injection or preputial application although one animal was infected orally by being dosed with infected tissue. They failed to infect rams by the subcutaneous, intravenous, conjunctival or nasal routes.

Infection by intratesticular injection was obtained by Kennedy *et al.* (1956), while Van Rensburg *et al.* (1958) infected rams by applying *Br. ovis* culture or infected semen to the glans penis of clean rams. Littlejohns and Lawrence (1959, unpublished) infected ram lambs by intravenous, intratesticular and preputial routes but could not demonstrate infection by the oral route.

Buddle (1956*a*) reported that rams between eight and 12 weeks of age could be infected by intravenous or subcutaneous inoculation although he thought that the disease might be less persistent than when older rams were infected. He was able (1954, 1956*a*) to infect older rams intravenously, orally, by injection into the testis and by preputial and conjunctival application of the organisms. Gdovin *et al.* (1956) could not infect immature rams.

Jebson *et al.* (1954) found that incubation periods for the development of genital lesions in rams after intravenous infection were usually six to eight weeks but preputial infection took seven to 17 weeks to produce lesions.

FERTILITY OF RAMS

The semen of infected rams is generally poor compared with that of normal rams and contains cells, sperm debris and often organisms of *Br. ovis* (Jebson *et al.*, 1954). Gunn *et al.* (1942) noted that seminal degeneration usually preceded the detection of lesions, and Osborne (1955) cites Gunn as finding semen in unilateral epididymitis to be grossly abnormal. Miller and Moule (1954) stated that when the history was available lowered fertility always preceded the development of palpable lesions. Snowden (1957) said that ovine brucellosis can affect the fertility of rams, although those with unilateral lesions might still be fertile, while McGowan (1958) examined semen from 29 natural cases and found definitely inferior semen in half of these but average quality or better in a quarter of them. Later McGowan and Devine (1960) showed that infected rams as a group had lowered fertility, but some individuals might be quite fertile. The assessment of the effect of epididymitis on fertility is difficult because of other factors that can cause infertility. McGowan and Shultz (1956) stated that rams with an incidence of epididymitis of 20 to 25 per cent showed much better fertility under mountain conditions in early autumn than when used in the valleys in summer. Clapp *et al.* (1957) attempted to separate the effects of epididymitis into two types: (*a*) where a large percentage of rams are infected with *Br. ovis* but lambing figures are good, and (*b*) where a small percentage of rams are infected but lambing figures are poor and are associated with abortions and dead lambs.

Edgar (1959) found that only two out of 15 rams with palpable unilateral epididymal lesions showed low fertility. He thought three semen examinations over six weeks were necessary before an adverse prediction could be given. Jebson *et al.* (1954) cite Crawford (1948) as saying that some palpably affected rams are fertile. However, Hartley *et al.* (1955) and Buddle (1955) found that actively infected Suffolk and Southdown rams running with clean Romney Marsh rams did not produce any crossbred lambs when all were mated with Romney Marsh ewes. Miller (1953) stated that test matings show that fertility is reduced by epididymitis. However, he did not consider that epididymitis caused a much lowered lambing percentage with flock matings unless the proportion of infected to normal rams was greater than one to ten.

INFERTILITY OF EWES

Although *Br. ovis* as a cause of abortion in ewes is well established its effect on

fertility is somewhat obscure. Snowden (1957) and Keogh *et al.* (1958) have produced some evidence to suggest that fertility in the ewe may be impaired.

DIAGNOSIS

Three methods have been used somewhat extensively in the diagnosis of ovine brucellosis. These are:

(*a*) genital palpation in the ram;

(*b*) complement fixation test on blood serum;

(*c*) stained smears and culture from semen and aborted foetal membranes.

Skin sensitivity tests with a formolized *Br. ovis* suspension were tried by Hall (1955), who found a correlation with the clinical state of rams, but because of technical difficulties this method of diagnosis has not been further investigated.

No one method appears to be very reliable for the diagnosis of disease in individual animals and even on a flock basis clinical examination should be combined with complement fixation tests and/or bacteriological examinations. In palpating rams' genitalia it should be borne in mind that epididymitis can be caused by other organisms than *Br. ovis*. Dodd and Hartley (1955) found abscess formation in the tail of the epididymis associated with an unidentified Gram-negative bacillus, and Jamieson and Soltys (1947) reported an epididymo-orchitis associated with *Pasteurella* pseudotuberculosis; Baynes and Simmons (1960) in Queensland described an organism that they called *Actinobacillus seminis* which can cause an epididymitis in sheep.

Naturally or experimentally infected rams do not invariably develop clinically detectable lesions although they may still be capable of disseminating highly infective semen over protracted periods (Buddle, 1955; Kennedy *et al.*, 1956; Biberstein & McGowan, 1958; Snowden, 1958). Kennedy *et al.* (1956) consider that a clinical latent period might extend over some months when only histological lesions are present; also early lesions may be difficult to detect, especially if bilateral, because of the variations in the normal epididymis (Hall, 1955).

Edgar (1959) examined 415 rams and found 42 with lesions but of these in only ten could he detect *Br. ovis*. He also found the organism in 29 rams with no palpable lesions. Miller and Moule (1954) observed that when spermatocoeles are present lesions are much easier to detect by palpation.

Edgar (1959) and Van Drimmelen (1959) both maintain that numbers of weakly acid-fast coccobacilli within neutrophils and epithelial cells in semen smears justify a diagnosis of infection, but according to Van Drimmelen it is common to find doubtful cases with only a few suspicious organisms present while semen samples from a ram infected for a period of some months may be positive one week and negative the next. Edgar thinks it is necessary to have three clear samples to give reasonable assurance that a ram is free from infection. Biberstein and McGowan (1958) concluded that only an unequivocally positive semen smear had any meaning and in the vast majority of cases doubtful or at best suggestive results are obtained. They, too, found that one ejaculate might contain large numbers of organisms while the next ejaculate from the same ram contained none.

If attempts are made to cultivate the organism from semen there is the risk of contamination with faster growing organisms normally present in or around the prepuce (Simmonds and Hall, 1953; Edgar, 1959). However Keats (1958, personal communication) found that pipetting the ejaculum from the bottom of the collecting tube into another sterile container immediately after collection reduced contamination to a minimum.

The complement fixation test has been used fairly extensively in Australia and also the U.S.A. and appears to be a useful aid to diagnosis especially on a flock basis. Clapp, Symons and Doolette (1955) obtained a good correlation between the complement fixation test and bacteriological examination, but not between the complement fixation test and clinical examination. Snowden (1958) also found that clinical lesions and complement fixation results did not agree very closely. Biberstein and McGowan (1958) in limited trials did not find very good correlation between complement fixation and the isolation of the organism or between complement fixation tests and clinical lesions.

METHODS OF CONTROL

Attempts to control ovine brucellosis have been made from two different approaches. In New Zealand a vaccine has been produced (Buddle, 1954, 1957*a*, 1958) and widely used in ram lambs. Vaccination consists of simultaneous injection of an adjuvant vaccine prepared from killed *Br. ovis* and living *Br. abortus* Strain 19. The *Br. ovis* vaccine alone gives a fair degree of protection, but the combined vaccine gives enhanced protection; Strain 19 alone will not give significant protection (Buddle, 1957*a*).

Vaccination of hoggets or older lambs may result in a temporary deterioration of semen quality for a period of two to nine weeks. This is a delayed effect which appears between two and five weeks after vaccination.

Strain 19 may be retained in the seminal vesicles of rams after it has disappeared from other parts of the body, including the inoculation site. It also produces antibodies which fix complement in the presence of the *Br. ovis* antigen (Anon., 1959). Gorrie (1959) found complement fixation titres persisting for at least two years in vaccinated rams; these were older than weaners when vaccinated.

Vaccination of ewes with either the combined vaccine or the adjuvant vaccine only, appears to increase the number of surviving lambs under experimental conditions. In field trials using the adjuvant vaccine alone the vaccinated group did have a considerably higher number of lambs than the controls (Buddle, 1958).

Because of the apparent venereal nature of the infection, the more transient nature of the disease in ewes, and their restricted role in the transmission of the disease, it seems to be unnecessary to vaccinate ewes as well as rams. Before young rams have been vaccinated they must be kept in isolation from older rams to prevent possible infection by sodomy.

Vaccination has not been encouraged in Australia and is prohibited in some states because it is thought that ovine brucellosis can be controlled and possibly eradicated from a flock by using the complement fixation test in conjunction

with clinical examination and/or semen examination, together with the isolation of young rams (Snowden, 1957). This method avoids the undesirable feature of vaccination, namely persistent complement fixation titres. Clapp *et al.* (1959) found it possible to remove infection rapidly from five out of eight flocks and to reduce it very considerably in the other three by complement fixation testing and slaughter of positives, before and after mating.

There are conflicting reports on whether the disease can be controlled by culling on palpation alone. Osborne (1955) says that this has been done, while McGowan (1958) says that new cases will be evident within a few months after culling. The success or otherwise of culling may depend on the type of mating that is carried out and whether complete segregation of young rams from old is maintained (Keogh *et al.*, 1958). These authors found that clean and infected flocks could be run on the same property without cross-infection for at least three years under Australian conditions, if the clean flock had first use of the yards and sheds.

THERAPY

Kuppaswamy (1954) appeared to have some success in the treatment of epididymitis due to experimental infection with *Br. ovis* of five months' duration. Treatment with chlortetracycline plus dihydrostreptomycin daily for 21 days resulted in apparent elimination of the organisms from the semen within a few days and resolution of the palpable lesions within the four months of the experiment. Dihydrostreptomycin alone or with sulphamezathine failed to give similar results. Kuppaswamy (1954) pointed out that the cost of treatment according to this régime would be excessive; it was not determined whether fertility, if impaired, was restored or not.

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AVIAN ENCEPHALOMYELITIS

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Although a considerable amount of work has been carried out on avian encephalomyelitis over the past 30 years, there is little precise information on the exact mode of transmission of the disease. Recently as a result of the application of new techniques to studies on the epidemiology and immunology of the disease, some new light has been thrown on the problem.

In France infectious avian encephalomyelitis was identified for the first time in 1958 and reported by Brion, Fontaine and Fontaine (1959). Since then the disease has increased in incidence and the present author has carried out studies on the pathology and epizootiology of the disease which have led to a hypothesis as to the nature and mode of transmission of the virus and which form the basis of this discussion.

NATURE OF THE VIRUS

The virus of infectious avian encephalomyelitis appears to be similar to poliomyelitis viruses, and is comparable in all ways with those already isolated from man and other animals. The size of the virus of avian encephalomyelitis is similar to that of other viruses within the group, being reported by Olitsky and Bauer (1959) to measure 20 to 30 millimicrons in diameter.

The histological changes produced in the nervous system are similar to those of polio-encephalomyelitis, and, as with poliomyelitic viruses in other species, changes are found in other organs such as the pancreas, heart, etc. This also occurs with the Coxsackie viruses. The lesions produced in the developing chick embryo are also comparable to some extent with those obtained with some strains of human poliomyelitic viruses, type 2 (Casorso & Jungherr, 1959), whilst recent work shows that the action of this virus in tissue culture is similar to that of the viruses of the poliomyelitic group (Hwang, Luginbuhl & Jungherr, 1959).

The virus of infectious avian encephalomyelitis is an enterovirus, as distinct from the viruses of the arbor group. Some workers have succeeded in isolating the virus from the droppings of both ailing and apparently healthy birds.

THE VIRUS IN ADULT BIRDS AND ITS TRANSMISSION BY THE EGG

Some workers have been able to produce nervous symptoms in adult birds, but only when large doses have been given by artificial routes of infection, and it is impossible from these experiments to determine the method of transmission from diseased to healthy birds under natural conditions producing clinically recognizable diseases.

Recent experiments have shown that apparently healthy adult birds may harbour virulent virus in the intestinal tract, that adult birds which have shown no clinical evidence of avian encephalomyelitis may produce eggs resistant to inoculation with the virus, and that antibodies can be transmitted in such eggs. Conversely, birds apparently of the same origin may at certain times lay infected eggs which in turn give rise to diseased chicks.

At first sight it seems difficult to explain why some hens transmit virus in their eggs and others antibody. This paradox is more apparent than real and the phenomenon may be explained in relation to the disease status during the first weeks of life, since a study of the nature of the virus indicates that infection occurs orally after hatching and the following possibilities may occur:

(1) Chicks already infected before hatching, but showing no symptoms, would have developed antibodies preventing subsequent development of the virus. Such birds, although they may be virus carriers, are themselves resistant and would lay eggs containing antibodies which in turn would produce healthy chicks.

(2) Chicks which are uninfected when hatched would lack antibodies and such birds would not exhibit nervous symptoms, for only infection of the embryo via the egg produces clinical disease. In such birds, two alternatives may occur: (*a*) either they will never come in contact with the virus, in which case the progeny will be uninfected but susceptible, or (*b*) they will become infected with the virus at a later stage and, although a short viraemia may occur, no clinical disease develops.

The time of infection therefore is important in determining the status of chicks hatched from their eggs.

If infection occurs before the birds come into production, they develop an immunity and will lay uninfected eggs containing antibody. The resultant progeny will be uninfected and resistant.

If infection occurs at the moment of egg production, eggs will be infected during a short period of viraemia. Generally speaking, however, the first eggs will be too small to be used for hatching and again there may be no infected chicks produced.

If, however, infection occurs after production has started and while the eggs from the infected birds are being used for hatching, the virus will be transmitted via the egg during the period of viraemia and clinical disease will appear in the hatched chicks. Once the viraemic phase has passed the infected birds become immune and transmit antibodies via their eggs.

A number of factors appear to influence the occurrence of inapparent infection in adult birds and it seems that the viraemic phase does not necessarily coincide with the time at which infection takes place. Included amongst these predisposing factors are:

(*a*) The neuroendocrine stress produced by egg production, which can be more serious in those birds which come into production before the completion of maturity;

(*b*) Intercurrent disease, both specific and non-specific, which reduces resistance;

(*c*) Various environmental factors. In fact, the drop in egg production which coincides with the laying of infected eggs is as likely to be due to extraneous causes as to the inapparent disease. The decrease in egg production referred to by many workers does not always occur and when it does it is often associated with the appearance of other diseases or with poor management.

(3) Chicks possessing antibodies when hatched receive only a small amount of maternal antibody via the egg and such birds when they mature will not transmit immunity in turn to their progeny. This fact would account for the periodic appearance of the disease in countries where it has become enzootic. In this way the entire avian population would become immunized in time and the virus would progressively disappear. Fully susceptible chickens would then reappear, capable of becoming infected and producing infected progeny.

Spread of the virus within the infected chick

Transmission of infection to the chick occurs solely via the egg from the infected ovary and there is no proof that there is any natural transmission of infection after hatching which is capable of producing the typical clinical disease.

It is common to find in infected flocks healthy but non-immune chicks in contact with clinically affected chicks, and yet such healthy chicks do not contract the disease even though mortality in the flock may be high. Furthermore, infection does not appear to take place during incubation and hatching, since if eggs of different origin are mixed in the same machine, only chicks hatched from infected eggs develop clinical disease.

It has been shown that the virus may be present in the yolk sac and the conclusion may be drawn, therefore, that the virus then invades the embryo. When one takes into account the anatomical differences between the chick and the mammal, the spread of the virus through the chick during its embryonic and post-embryonic development can be explained on the basis of Fenner's theory (1956) on the mode of spread of enteroviruses. Since the virus is present in the yolk sac it is transferred into the digestive tract of the embryo during the absorption of the yolk sac and continues its multiplication in the intestinal tract. It is not yet known by what method the virus penetrates the intestinal barrier and gains access to the embryonic tissues, either by the lymphatics or the blood stream. However, a sudden viraemia appears, the virus affecting many organs. Spread may occur by successive steps and since the bird has no lymph nodes, the lymphoreticular system may restrain or even stop virus multiplication.

Occasionally the virus is able to overcome the resistance of the nervous system and produce clinical changes, and the appearance of ataxia and tremors in avian encephalomyelitis may be coincidental.

Histological and epizootiological studies appear to confirm the point of view that in naturally occurring avian encephalomyelitis, lesions occur in both nervous and visceral organs. Conversely, when the virus is artificially inoculated intracerebrally, where it is most pathogenic, no visceral lesions are produced. But in both the natural and in the experimental form of the disease when the

virus is inoculated by the parenteral route, it spreads throughout the tissues before reaching the nervous system and produces lesions in the different tissues through which it passes. Furthermore, the spread of the virus seems to be largely by the blood stream since the lesions are always found near or in the blood vessels (except in the spinal cord). Moreover it has been noted by a number of workers that in a group of chickens affected with this disease apparently healthy birds may show lesions. An analysis of the distribution of these lesions shows that those of the visceral organs, particularly the pancreas, are the commonest, whereas lesions in the nervous system may be few or even absent. This indicates that the virus has multiplied within the body without invading the nervous system, and even if the virus does reach the nervous system the lesions are so mild that clinical symptoms are absent.

In field outbreaks the number of birds showing symptoms is unimportant and it would appear that other factors which reduce the resistance of the chick enable the virus to penetrate the natural defence barriers and reach the nervous system, resulting in an increase in the number of affected chicks. The factors concerned are transport stress, poor hygiene, malnutrition, etc. In these field cases nervous symptoms appear between the second and fourth weeks after hatching.

In the experimentally-produced disease the incubation period is slightly longer, even when large doses of virus are given by routes of inoculation which bring the virus directly into contact with the nervous system.

All these facts seem to prove that multiplication of the virus originates in the embryo during incubation and that infection occurring after hatching never produces the clinical disease under natural conditions. The virus continues to multiply after hatching and may sometimes reach the nervous system where it produces lesions and characteristic symptoms of the disease. These facts can be confirmed by field observations on flocks affected with avian encephalomyelitis. In fact, although encephalomyelitis may be diagnosed clinically and histologically in birds of two to four weeks of age, it may be impossible categorically to confirm the disease in younger chicks from the same flock which have died without having shown clinical symptoms and in which no specific histological changes can be found. It is reasonable to conclude that an acute form of encephalomyelitis has occurred and that there has been no time for reactive lesions to occur in the tissues. This theory would indicate that all organs have been simultaneously acutely infected by the virus and would also explain the increase in embryonic mortality which occasionally occurs.

Experimentally the virulence of virus strains isolated from spontaneous cases varies considerably. This may be due to several causes:

(1) Difficulty in isolating the virus from nervous tissues. This may be due to the fact that (*a*) the "encephalitic reaction" can eliminate the virus completely ("neuro-infection mortelle auto-sterilisable" of Levaditi [Levaditi, Lepine & Schoen, 1929]), or (b) the virus may be so intimately bound to the nerve cell that it is impossible to separate them. Methods of preparation of the inoculum such as that indicated by Feibel, Helmboldt, Jungherr and

Carson (1952) are therefore advantageous.

(2) Difference in the susceptibility of experimental chickens to artificial infection with the virus.

(3) The existence of different strains of the virus. Strains of the virus isolated do not seem to possess the same pathogenicity; some show a light neurotropism but strong viscerotropism. This would appear to be the case, for example, in a strain isolated by Markson and Blaxland (1958). During successive passages in susceptible chickens all strains showed an increase in neurotropism. As a result of this adaptation of the virus to nervous tissue the incubation period is shortened and the morbidity of the disease increased.

It is always difficult to reproduce nervous symptoms in chicken by routes other than the intracerebral. The oral route of inoculation gives negative results, which accounts for the failure to reproduce natural infection in birds of all ages. It is almost impossible to obtain positive results in embryonated eggs by any route of inoculation other than intra-yolk, intracerebral or intraocular. When the virus multiplies in the egg it is only after hatching that clinical symptoms appear.

The geographical spread of infectious avian encephalomyelitis

The virus can be considered to belong to the group of enteroviruses and is transmitted in two ways: (1) the horizontal route, which occurs after hatching when infection is transmitted by the digestive route and does not result in clinical disease; (2) the vertical route, by which infection is transmitted via the ovary and results in the clinical appearance of disease in the hatched chick. Gear (1958) questioned whether infection by this route (similar to uterine infection) occurred with Coxsackie virus.

The epizootiological pattern of avian encephalomyelitis in any country would appear to be comparable with that of human poliomyelitis. This is easier to understand in avian encephalomyelitis since birds live in flocks with access to their own excreta. Therefore a situation arises where the spread of infection is rapid and complete, with subsequent transmission of antibodies via the egg (in the bird the yolk sac takes the place of the human colostrum).

The progress of the disease has therefore to be considered from two aspects, i.e. within a breeding flock and on a geographical basis.

Until recently Europe could be considered to be free from this disease. It would appear that the virus originated in imported birds and it became widespread as a result of breeders selling such stock over a wide area. The incidence of the disease has increased considerably in France since 1958 and no longer shows a marked seasonal incidence. It is to be anticipated that avian encephalomyelitis will disappear progressively, for in time the whole avian population will be infected. Periodically it may reappear in some countries as a result of the virus having disappeared because of the development of immunity in birds and therefore the re-establishment of sensitive populations.

The same epizootiology apparently occurs within a breeding flock. For example, if infected breeding stock (especially males) are introduced into a healthy flock, infectious avian encephalomyelitis will appear in chickens hatched

from the infected hens and subsequently in chicks hatched from birds in adjacent pens. Eventually the entire avian population of the farm becomes infected, breeding birds develop an immunity which they transmit to their progeny and the disease disappears.

Conversely, in infected flocks the disease occurs in the progeny hatched from newly imported susceptible hens or from hens which have lost their immunity. In such cases the disease will appear only in the chicks hatched from that particular pen. This may lead the farmer to believe that infection was introduced by the new birds, but this is not the case and these newly-introduced susceptible birds merely show up the latent infection present in the flock.

DISCUSSION

Although the conclusions advanced are theoretical, they are nevertheless based on actual evidence. They have the merit, moreover, of being comparable to the views held in regard to other diseases of similar pathogenicity, e.g. poliomyelitis and Teschen disease in swine (Mayr & Hecke, 1960), and are therefore of use in developing more efficient control measures. In infectious avian encephalomyelitis, the usual methods of sanitary control are ineffective. Certainly they are in infected countries, for there is no known means of detecting carriers of the virus. Vaccination, however, offers some hope of success, provided that it is systematically carried out before the flock are in production and therefore capable of transmitting the vaccinal virus to the progeny.

Unfortunately at the moment there is insufficient knowledge concerning the nature of the virus and its transmission and as there may be several strains of virus serologically different one from the other, vaccination in one country may not be effective in another.

The alternative to passively waiting until the virus has infected the whole avian population of the country may be even more dangerous than the policy of vaccination.

SUMMARY

Hypotheses are advanced regarding the epizootiology of infectious avian encephalomyelitis, based on experimental and field evidence.

Attention is drawn to the similarity which exists between the transmission of this disease and the horizontal and vertical methods of transmission ascribed to other viruses. In infectious avian encephalomyelitis, however, the horizontal cycle does not result in clinically recognizable disease but it produces carriers and subsequently immune birds. It is the vertical transmission of the disease via the infected eggs from carrier birds which produces clinical infection in the progeny. Immune birds, however, can transmit antibodies to their progeny.

The pathogenesis of the disease within the individual bird and chick and the epizootiological pattern within a flock and geographical area are discussed in some detail. It is concluded that at the moment control by the detection and elimination of carriers is impossible and that vaccination of birds prior to egg production offers the most hopeful solution. The only alternative appears to be to permit the disease to become enzootic until the virus has infected the entire avian population of an area and produced an immunity.

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THE EFFECTS OF HORMONE IMPLANTATIONS ON THE GROWTH RATES OF EUROPEAN, ZEBU AND HYBRID BEEF STEERS GRAZING IN JAMAICA

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Many workers have reported that the subcutaneous implantation of stilboestrol may improve the growth rate and feed efficiency of fattening beef cattle. Most of these reports have originated from the United States (Clegg and Cole, 1954), where the oral administration of stilboestrol has also received considerable attention (Burroughs *et al.*, 1954, 1955; Perry, Beeson, Andrews & Stob, 1955). In the United Kingdom hexoestrol is preferred to stilboestrol, since it is believed to be less active if accidentally ingested by humans. Aitken and Crichton (1956) and Burgess and Lamming (1960) have reported significant responses to hexoestrol implanted into steers in Britain.

Although most of the published work on this subject has been carried out with animals which were fed concentrates, reports from Australia (Tulloch, 1959), New Zealand (Everitt, 1959), the Argentine (Callow and Finney, 1959) and Tanganyika (Anon., 1960) describe responses in grazing steers implanted with hexoestrol. Similar responses were obtained when stilboestrol was implanted into grazing steers in the U.S.A. (Koch, Gregory, Ingalls and Arthaud, 1959).

A wide range of doses of hexoestrol implants has been used, responses having been reported with doses from 15 to 120 mg. Some of the more recent papers (Everitt, 1959; Burgess and Lamming, 1960; Callow and Finney, 1959) suggest that the optimum response is obtained from doses of about 30 mg. which give a similar growth effect to the higher doses, while at the same time they reduce the undesirable side effects such as elevation of the sacrum and coccygeal vertebrae and excessive development of mammary tissue.

It has been suggested that the quality and nature of the roughage portion of the diet significantly influences the response to hormone treatment. Aitken and Crichton (1956) obtained a response in steers given a high roughage ration, but on this ration the weight gains of the controls exceeded 1 lb./day. Quinn, Mott, Bischoff and McClung (1958) and Callow and Finney (1959) obtained responses in implanted steers on natural pasture where the control groups gained a little less than 1 lb./day.

Most of the published work has described experiments carried out with European beef breeds, but the papers from Tanganyika (Anon., 1960) and from Quinn *et al.* (1958) describe work done with Zebu steers implanted with hexoestrol and stilboestrol respectively.

The purpose of the work described in this paper was to assess the value of implantation of both hexoestrol and a progesterone-oestradiol compound (implant A; Synovex, Squibb) given to grazing steers. The experiments were carried out on commercial farms which, in accordance with local practice, fed no concentrate supplement. Experiments were also designed to study:

(1) the relative merits of hexoestrol and implant A;

(2) the optimum dose of hexoestrol;

(3) the effect of implantation on the dressing percentage of the carcase;

(4) whether there was any correlation between breed type (pure Zebu, high-grade European and hybrid) and hormone response;

(5) whether the response was the same when animals of various sizes were implanted;

(6) whether the plane of nutrition affected hormone response.

METHODS

Experiments were carried out on pasture in different seasons in 1958, 1959 and 1960 at five farms. On three of them the steers grazed Guinea Grass (*Panicum maximum*) and on the other two the grazing was Pangola Grass (*Digitaria decumbens*). All steers were implanted subcutaneously on the upper surface of the ear. Hexoestrol pellets of 15 mg. each were inserted from a poultry implanter. Implant A pellets were implanted from a special machine which administered at one time six pellets containing a total dose of 200 mg. progesterone and 20 mg. oestradiol benzoate.

Steers were allocated to groups according to a stratified random scheme which balanced groups according to live weight. In experiments 4-6, groups were also balanced according to breed and in these experiments steer pairs were analysed as blocks. All steers were acclimatized to their trial environment by grazing on it for at least three days prior to being weighed for the experiment. Weighing was thereafter carried out monthly in the mornings without any overnight starvation. Starved weights were obtained prior to slaughter in experiments 2 to 4.

Carcase evaluation was somewhat empirical since at the time of these experiments only two grades of beef were recognized locally.

Statistical analyses were carried out as described by Snedecor (1956).

Experiment 1

Experiment 1 was carried out in August 1958 with high-grade Angus steers. Five were implanted with implant A at an average live weight of 760 lb. and five similar steers acted as controls. The experiment had to be restricted to four months' duration because of movement restrictions imposed by disease control regulations. During the period of the experiment the farm was experiencing a drought and pasture conditions varied considerably from month to month.

Experiments 2 *and* 3

These two experiments were carried out in March-June and March-August 1958, in a dry area with poor quality Guinea Grass grazing.

Experiment 2 was designed to test the effect of 60 mg. hexoestrol implants in Angus and Red Poll steers. Ten out of a group of 20 steers of nearly 1,000 lb. live weight each were implanted and grazed for 118 days before slaughter. Full and starved dressing percentages and carcase quality were recorded.

Experiment 3 was similar in design to No. 2 except that implant A was used on nine steers, each of 900 lb. live weight, and these animals were grazed for 161 days before slaughter.

Experiment 4

Forty-four Angus and Red Poll steers were paired for type and weight in October 1959 and one member of each pair was implanted with 60 mg. of hexoestrol. The animals averaged about 850 lb. at the time of implantation. They were rotationally grazed on lightly fertilized Pangola Grass, as one herd, for 95 days following implantation. Weights were recorded at approximately monthly intervals. At the end of the 95 days, live-weight gains of all steers were recorded and 12 control and seven treated steers were slaughtered and carcase quality and dressing percentage noted.

Experiments 5 *and* 6

These two experiments were designed to test the effects of hexoestrol and implant A when implanted in steers rotationally grazing high quality Pangola Grass. All steers were typed as either European or hybrid and the experiments were designed so that an analysis of variance for type effect was possible. In both of these experiments the steers were implanted at store weights and were sold for fattening.

In experiment 5, carried out from September-November 1959, 84 steers were selected, 42 from each of two pangola pasture groups (fertilized and unfertilized), and randomly divided according to weight and type into six groups, each of 14 steers. Two groups were implanted with 60 mg. hexoestrol and two with implant A. Live-weight gains were recorded for two months, during which time the animals were being prepared for sale. Since the design of the pasture experiment provided that the two pasture groups should be stocked to maintain a similar daily live-weight gain in steers in both lots, and

a test of agreement showed that this requirement was satisfied, the two replicates have been pooled in the tabulated results.

Experiment 6 followed the same pattern as No. 5 except that implant A was not used and two groups, each of 20 steers, were implanted in January, 1960 at 540 lb. live weight and sold 84 days afterwards.

Experiments 7 and 8

A fattening lot of 100 steers was classified in May, 1960, as 24 European, 20 pure-bred Zebu (Mysore) and 56 hybrids. It was not possible to pair each group in a balanced manner, but a random 12 Europeans, 10 Zebus and 14 hybrids were implanted with 60 mg. hexoestrol doses (Experiment 7). Fourteen hybrids were then implanted with 45 mg. and 14 with 30 mg. hexoestrol (Experiment 8).

All steers grazed average quality Guinea Grass for 119 days, after which they were again weighed and live-weight gains tabulated.

RESULTS

Implant A and live weight

Implant A was used in three experiments (Nos. 1, 3 and 5). Only in Experiment 5 was there a significant difference which could be attributed to treatment with this hormone (Table I).

TABLE I
THE EFFECT OF IMPLANT A ON THE LIVE-WEIGHT GAINS OF GRAZING BEEF STEERS

	Expt. 1		*Expt.* 3		*Expt.* 5	
	Control	*Implanted*	*Control*	*Implanted*	*Control*	*Implanted*
Number of steers	5	5	10	10	28	28
Duration of expt. (days)	119	119	161	161	56	56
Average initial weight (lb.)	716	769	903	897	615	612
Average gain (lb.)	130	175	170	187	77	87
Average daily gain (lb.)	1·09	1·47	1·06	1·16	1·38	1·56*
S.E. of daily gain	0·34		0·30		0·30	
% increase attributed to implant	35		9		13	

* Difference significant at $P<0.05$

Hexoestrol (60 mg.) and live weight

In five experiments (Nos. 2, 4, 5, 6 and 7) the dosage used was 60 mg. of hexoestrol. In all of them the implanted group gained more in live weight than did the control steers during the treatment period. In Experiments 4 and 5 this increase was significant at the 1 per cent level of probability, and in Experiment 6 it was significant at the 0·1 per cent level. In Experiments 2 and 7 the implanted steers gained 20 per cent and 24 per cent, respectively, more than their untreated partners. It is probably due to the fact that the design of these two experiments did not permit of pairing to reduce variation for sizes and types of steers that these last two results were not statistically significant. Table II displays a summary of the responses to hexoestrol treatment.

TABLE II

THE EFFECTS OF IMPLANTS OF 60 MG. HEXOESTROL ON THE LIVE-WEIGHT GAINS OF GRAZING BEEF STEERS

	Experiment 2		*Experiment 4*		*Experiment 5*		*Experiment 6*		*Experiment 7*	
	Control	*Implanted*	*Control*	*Implanted*	*Control*	*Implanted*	*Control*	*Implanted*	*Control*	*Implanted*
Number of steers	9	9	22	21	28	28	40	40	36	36
Duration of experiment (days)	118	118	95	95	56	56	84	84	119	119
Average initial weight (lb.)	990	982	826	823	615	615	539	538	648	663
Average gain (lb.)	95	118	59	107	77	97	118	139	107	128
Average daily gain (lb.)	0·81	1·00	0·62	1·13*	1·38	1·72*	1·40	1·65†	0·90	1·07
S.E. of daily gain	0·58		0·30		0·32		0·24		0·38	
% increase attributed to implant	24		80		25		18		20	

* Difference significant at $P<0.01$

† Difference significant at $P<0.001$

In Experiment 6 the steers rotationally grazed a series of lush pangola pastures and it was possible to carry out a series of monthly weighings during this experiment in order to follow the rapidity of the response to implanted hexoestrol. The response became apparent within 28 days and persisted for at least 84 days (Table III).

TABLE III

ANIMAL GAINS (IN LB.) ATTRIBUTED TO HEXOESTROL

	Control	*Hexoestrol*	S.E.
Number of steers	40	40	
Average gain in 28 days (lb.)	46	55*	17·89
Average gain in 56 days (lb.)	90	105†	19·57
Average gain in 84 days (lb.)	118	139‡	20·23

* Difference significant at $P<0.05$
† Difference significant at $P<0.01$
‡ Difference significant at $P<0.001$

Dosage of Hexoestrol

Experiment 8 was designed to test the effects of different dosage levels of hexoestrol on the subsequent gains of grazing hybrids. There was little difference between the responses of the groups receiving 30, 45 and 60 mg. hexoestrol. The animals were assigned to treatments in groups of 14 and not allocated in blocks, and the large within-treatment variation was again the probable explanation of the lack of a significant response. The 30 mg. dose produced the least side effects and the results (Table IV) suggest that this dose is adequate.

TABLE IV

THE EFFECT OF VARIABLE DOSES OF HEXOESTROL ON THE SUBSEQUENT LIVE-WEIGHT GAINS OF GRAZING HYBRID STEERS

	Dose of hexoestrol (mg.)				
	0	30	45	60	S.E.
Number of steers	14	14	14	14	
Duration of experiment (days)	119	119	119	119	
Initial weight (lb.)	649	656	696	674	
Average gain (lb.)	107	142	132	132	
Average daily gain (lb.)	0·90	1·19	1·11	1·11	0·44
% increase attributed to implant		32	23	23	—

Dressing percentage

In Experiments 2, 3 and 4 full and starved farm weights were obtained prior to slaughter. In Experiment 4, data were available for only a random 19 of the steers slaughtered. In the other two trials all steers were slaughtered. In all three groups the implanted steers dressed at a slightly lower percentage on a starved farm weight, although in only one case did the treated group dress lower on a "full" basis. It would therefore appear as if there was a slightly greater loss of weight prior to slaughter in implanted steers.

There was no apparent difference in the nature of the dressing percentages between the two types of hormone implants (Table V).

TABLE V

THE EFFECTS OF HORMONE IMPLANTATION ON THE DRESSING PERCENTAGE OF EUROPEAN STEERS SLAUGHTERED AFTER GRAZING

	Experiment 2		*Experiment 3*		*Experiment 4*	
	Control	*Hexoestrol*	*Control*	*Implant A*	*Control*	*Hexoestrol*
Number of steers	9	9	10	10	12	7
Average full final wt. (lb.)	1,085	1,100	1,063	1,084	911	966
Average starved final wt. (lb.)	1,003	1,042	1,034	1,053	894	887
Average warm dressed carcase wt. (lb.)	578	591	576	573	488	517
Dressing as % fed final wt.	53·3	53·7	54·3	52·8	53·5	53·6
Dressing as % starved final wt.	57·6	56·7	55·7	54·4	56·3	58·3

Breeding

In Experiment 6, all steers were Angus, Red Poll, or produced by crossing cows of these breeds with Zebu bulls. There were only seven pairs of Angus steers; when these were discounted there remained 33 pairs of either Red Poll or hybrid steers. An analysis of variance was carried out to study the effect of breed type and hormone. There was a highly significant ($F < 0{\cdot}001$) response to both hormone treatment and to breed type (hybrids surpassing the Red Polls), but there appeared to be no interaction between the two. Table VI shows the importance of the superior growth of the hybrids and emphasizes how faulty steer pairing may distort the interpretation of results when experiments are conducted under ranching conditions.

TABLE VI

THE EFFECTS OF BREEDING ON RESPONSES TO IMPLANTED HEXOESTROL (60 MG.)

	Red Poll		*Hybrids*		
	Control	*Hexoestrol*	*Control*	*Hexoestrol*	S.E.
Number of steers	16	16	17	17	
Duration of experiment (days)	84	84	84	84	
Average initial weight (lb.)	539	534	543	542	
Average gain (lb.)	111	125	128	148	
Average daily gain (lb.)	1·32	1·49*a	1·52*a	1·76*abc	0·26

* Difference significant at $P < 0{\cdot}001$
a = compared with control Red Polls
b = compared with control hybrids
c = compared with hexoestrol-implanted Red Polls

In Experiment 7 steers were paired as Zebus, Europeans and hybrids. The Zebus were a very uniform group from a closed herd and gave a highly significant response to hexoestrol implantation. The hybrids had a much superior daily gain when compared with the Zebus, but within the hybrid group there was a large standard error and the increased gain due to hexoestrol was not significant. The European group contained very variable types of animals which could not be paired satisfactorily and the two groups of European types made similar weight gains. Table VII is of interest in that it shows the superior growth rate of the hybrids and the degree of response in the Zebus where the experimental design minimized errors due to breeding, weight and nutrition.

In Experiment 6 the steers rotationally grazed a series of lush pangola pastures and it was possible to carry out a series of monthly weighings during this experiment in order to follow the rapidity of the response to implanted hexoestrol. The response became apparent within 28 days and persisted for at least 84 days (Table III).

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	Dose of hexoestrol (mg.)				
	0	30	45	60	S.E.
Number of steers	14	14	14	14	
Duration of experiment (days)	119	119	119	119	
Initial weight (lb.)	649	656	696	674	
Average gain (lb.)	107	142	132	132	
Average daily gain (lb.)	0·90	1·19	1·11	1·11	0·44
% increase attributed to implant		32	23	23	—

Dressing percentage

In Experiments 2, 3 and 4 full and starved farm weights were obtained prior to slaughter. In Experiment 4, data were available for only a random 19 of the steers slaughtered. In the other two trials all steers were slaughtered. In all three groups the implanted steers dressed at a slightly lower percentage on a starved farm weight, although in only one case did the treated group dress lower on a "full" basis. It would therefore appear as if there was a slightly greater loss of weight prior to slaughter in implanted steers.

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Average warm dressed carcase wt. (lb.)	578	591	576	573	488	517
Dressing as % fed final wt.	53·3	53·7	54·3	52·8	53·5	53·6
Dressing as % starved final wt.	57·6	56·7	55·7	54·4	58·5	58·3

Breeding

In Experiment 6, all steers were Angus, Red Poll, or produced by crossing cows of these breeds with Zebu bulls. There were only seven pairs of Angus steers; when these were discounted there remained 33 pairs of either Red Poll or hybrid steers. An analysis of variance was carried out to study the effect of breed type and hormone. There was a highly significant ($P<0.001$) response to both hormone treatment and to breed type (hybrids surpassing the Red Polls), but there appeared to be no interaction between the two. Table VI shows the importance of the superior growth of the hybrids and emphasizes how faulty steer pairing may distort the interpretation of results when experiments are conducted under ranching conditions.

TABLE VI

THE EFFECTS OF BREEDING ON RESPONSES TO IMPLANTED HEXOESTROL (60 MG.)

	Red Poll		*Hybrids*		
	Control	*Hexoestrol*	*Control*	*Hexoestrol*	S.E.
Number of steers	16	16	17	17	
Duration of experiment (days)	84	84	84	84	
Average initial weight (lb.)	539	534	543	542	
Average gain (lb.)	111	125	128	148	
Average daily gain (lb.)	1·32	1·49*a	1·52*a	1·76*abc	0·26

* Difference significant at $P<0.001$
a = compared with control Red Polls
b = compared with control hybrids
c = compared with hexoestrol-implanted Red Polls

In Experiment 7 steers were paired as Zebus, Europeans and hybrids. The Zebus were a very uniform group from a closed herd and gave a highly significant response to hexoestrol implantation. The hybrids had a much superior daily gain when compared with the Zebus, but within the hybrid group there was a large standard error and the increased gain due to hexoestrol was not significant. The European group contained very variable types of animals which could not be paired satisfactorily and the two groups of European types made similar weight gains. Table VII is of interest in that it shows the superior growth rate of the hybrids and the degree of response in the Zebus where the experimental design minimized errors due to breeding, weight and nutrition.

TABLE VII

THE EFFECTS OF HEXOESTROL (60 MG.) ON THE SUBSEQUENT LIVE-WEIGHT GAINS OF VARIOUS TYPES OF FATTENING STEERS

	Zebus		*Europeans*		*Hybrid*		*All groups*	
	Control	*Hexoestrol*	*Control*	*Hexoestrol*	*Control*	*Hexoestrol*	*Control*	*Hexoestrol*
Number of steers	10	10	12	12	14	14	36	36
Duration of expt. (days)	119	119	119	119	119	119	119	119
Average initial wt. (lb.)	620	622	671	682	649	674	648	663
Average gain (lb.)	81	116	129	131	107	132	107	128
Average daily gain (lb.)	0·68	0·98*	1·08	1·10	0·90	1·11	0·90	1·07
S.E. of daily gain	0·18		0·23		0·48		0·38	

* Difference significant at $P<0.001$

DISCUSSION

The results of the experiments reported here suggest that an increase in live-weight gain can be produced in grazing steers implanted with hexoestrol and maintained on Jamaican pastures.

The carcase studies show a tendency for implanted steers to dress slightly lower than controls but not significantly so. This trend has been noticed by Burgess and Lamming (1960) and by Klosterman, Cahill, Kunkle & Moxon (1955). There was no difference in carcase quality in treated steers when carcases were assessed by local standards. This observation is in agreement with the studies of Aitken and Crichton (1956) and Burgess and Lamming (1960) in the United Kingdom, and those of Cahill, Kunkle, Klosterman, Deatherage & Wierbicki (1956) and O'Mary, Warren, Davis & Pierce (1956) in the U.S.A. Other American authors (Jordan, 1953; Andrews and Beeson, 1953; Clegg and Cole, 1954; Perry *et al.*, 1955) have claimed that hormone treatment reduces carcase quality. This is probably due to there being less fat covering over the carcase, an attribute that is not important for the Jamaican market.

Elevation of the sacrum and coccygeal vertebrae and enlargement of the rudimentary teats were two side effects that were obvious in many of the animals implanted with 60 mg. hexoestrol. These effects were less obvious in animals given implant A and also in those given smaller doses of hexoestrol. The fact that 30 mg. of hexoestrol in the last experiment gave an effect similar to that produced by 60 mg. appears to support the case made by Burgess and Lamming (1960) and Everitt (1959) for the use of smaller doses (30 mg.) of hexoestrol.

Table VIII shows that hexoestrol was used in steer groups ranging from 550 to 1,000 lb. in weight. We were unable to detect any association between animal live weight and hormone response within any of these experiments. All of these steers were aged between 18 months and three years – the age range in which most store and fat cattle are marketed in Jamaica. In this series significant responses were obtained in treatment groups where control gains ranged from 0·62 to 1·40 lb. live weight per day. Most grass-fattened steers in Jamaica have daily gains within this range. In the experiments with the lowest daily

TABLE VIII

A SUMMARY OF THE EFFECTS OF HEXOESTROL IMPLANTATION ON STEERS OF VARYING WEIGHTS

Experiment No.	*Grazing quality*	*Number of treated steers*	*Approx. initial wt. (lb.)*	*Period of experiment (days)*	*Gain per day*		*% extra gain from treatment*	*Significance*
					Control (lb.)	*Treated (lb.)*		
2	Fair	9	1,000	118	0·81	1·00	24	None
4	Fair	21	800	95	0·62	1·13	80	$P<0·01$
5	Good	28	600	56	1·38	1·72	25	$P<0·01$
6	Good	40	550	84	1·40	1·65	18	$P<0·001$
7	Fair	36	650	119	0·90	1·07	20	None

gains the steers were grazing Guinea Grass of high fibre content. This did not appear to inhibit the action of the hexoestrol.

In most cases where a response was obtained, it appeared to be of the order of a 20 per cent increase in live weight, or 20 to 30 lb. in a three to four-month period. These observations are not necessarily applicable to the results from using implant A where there is a suggestion that the implant fails to give a significant response where live-weight gains are less than 1·4 lb./day.

The fact that the hexoestrol response does not in any way appear to be associated with the proportion of either *Bos taurus* or *Bos indicus* breeding is encouraging, since a great deal of crossing between these two breeds occurs in Jamaica. The one hexoestrol experiment (No. 8) with pure Zebu (Mysore) steers aged two and three years gave a daily gain in the treated and control groups of 0·98 lb. and 0·68 lb., respectively. This is in very close agreement with the work of Quinn *et al.* (1958) who obtained in two-year-old Zebu implant and control groups gains of 1·13 and 0·79 lb./day, and in three-year-olds 1·09 and 0·69 lb./day, respectively. A range of results was obtained with European and with hybrid steers but in Experiments 5 and 6 it was not possible to show significant differences between the responses of these two types to implantation. Since the majority of beef cattle in Jamaica are on the one hand either planned *Bos indicus* × *Bos taurus* hybrids, or on the other hand result from a deliberate attempt to breed a tropically adapted strain of European Red Poll or Angus cattle, it is of some practical value to know that the hormone gives a similar response in both types.

Although the phenotypic basis of classifying cattle as European or hybrid is open to criticism, it is an accepted criterion of the large beef farmer with a cross-breeding programme, and on such a basis the results of experiments with the two groups assume a practical significance.

The economic implications of implantation also require comment, in that at current beef prices, a live-weight gain of 27 lb. attributed to implantation represents a profit of £2 over and above the cost of implantation with hexoestrol. With implant A the profit is about 30 per cent lower (£1 8s. 0d.) because of the increased cost of the implant. On a very high plane of nutrition the increased gains induced by this latter implant may make its use economic, but under the conditions of this experiment, which are typical of the better type of beef farm in Jamaica, the use of implant A was not economical.

SUMMARY

The use of hexoestrol and of a progesterone/oestradiol compound implanted subcutaneously into beef steers grazing farm pastures has been studied. Doses of hexoestrol (60 mg.) gave increased live-weight gains in all of five series of experiments. These gains were usually of the order of an increase of about 20 per cent over the gain made by control steers. They were statistically significant at the 1 per cent level in three out of five experiments. By local criteria, no change in carcase quality was induced by hexoestrol, but there was a small non-significant fall in dressing percentage. Hexoestrol in 60 mg. doses produced noticeable side effects which were less marked in animals receiving implants of 45 and 30 mg., at which dosage levels the growth response to hexoestrol was not diminished.

The response to hexoestrol was independent of the breeding of the steers (*Bos indicus*, *Bos taurus* and hybrids), the size of the steers (550-1,000 lb.), the pasture quality (live-weight gains of 0·62 to 1·60 lb./day) and the dose of hexoestrol (30-60 mg). There was an interaction between live-weight gain and breeding (hybrids surpassed high grade *Bos taurus* steers) in two experiments. This may have been the reason for the failure to obtain a significant response in experiments where accurate pairing on a type basis was not done.

Implantations of progesterone plus oestradiol in these experiments gave disappointing results which suggested that the compound was of limited value under the grazing conditions encountered. In one comparison with hexoestrol where the progesterone-treated steers had a significant response with a daily gain of 1·56 lb., the hexoestrol-treated steers surpassed them in daily gain.

From the economic standpoint the use of the progesterone implant in grazing steers was uneconomic but hexoestrol implantation appeared to be highly profitable.

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SOME ASPECTS OF AGRICULTURAL RESEARCH: No. 2.

Review of The Report of the Agricultural Research Council for the year 1958-59*.

By T. Dalling
Edinburgh

The articles contained in the annual reports of the Agricultural Research Council are always varied and topical. It is an excellent idea that they should be published apart from the report as a collection of papers. No. 2 of the series provides interesting reading to those working on different subjects. To veterinary readers the articles on foot-and-mouth disease and metabolic diseases of ruminants will, naturally, be considered the most important; that on blood types in cattle, however, also has a definite appeal to veterinarians. The other papers concern research and the apple industry; plant breeding; losses in crops from pest and diseases; some aspects of plant research; and the quality of milk.

FOOT-AND-MOUTH DISEASE

This article deals with many aspects of the control of foot-and-mouth disease and recent research work carried out mainly at the Research (Animal Viruses) Institute, Pirbright, Surrey. In the review, reference is made to the report of the Departmental Committee under the chairmanship of Sir Ernest Gowers in which full support was given to the slaughter policy operated in this country for control of the disease, and it is stated that these decisions "have been fully justified by events since 1954". While this policy can well be applied in this country it is stated that it is not generally applicable, especially in those parts of the world where the disease is endemic. The "realistic" overall plan of the European Commission for the Control of Foot-and-Mouth Disease is given in full. It consists of successive stages of control, beginning with highly infected countries, whereby by systematic vaccination against the disease, eventually the position may be reached when a stamping-out policy can be entirely relied upon. It is very rightly stated that in any slaughter policy there must be included many other precautionary measures, such as early reporting of outbreaks, tracing of animals which may have been exposed, satisfactory disposal of the carcases of slaughtered animals, the application of immediate and severe animal movement restrictions, adequate disinfection procedures and "considerable elasticity in financial arrangements to allow of vigorous pursuit of the policy".

* London: H.M. Stationery Office 3/6.

Attention is drawn to the importance of typing the strains of the causal virus in outbreaks and to the existence of the three African types and the Asian type, at present confined to regions in Africa and Asia, respectively, all immunologically different from the three "classical" strains found in other parts of the world and from each other: these four types were first recognized at the Pirbright Institute.

Much typing work is carried out at the Institute, the technique used depending on circumstances: some 80 per cent of virus in samples from outbreaks in Great Britain have been successfully typed by the relatively simple complement fixation test and results are available within a matter of hours. On the other hand, other methods have to be used for some samples, especially for samples received from abroad. The discovery of the high susceptibility of unweaned mice at the Pirbright Institute has led to their use in some of the typing work.

The Pirbright Institute has been appointed the World Reference Laboratory and is giving valuable service to other countries in typing, in the provision of antisera for typing purposes in other laboratories and in carrying out epidemiological surveys in different parts of the world. This latter work has much bearing on methods of control and on risks associated with the movements of animals and with trade in animal products. The responsibilities at the World Reference Laboratory also include the classification of types and sub-types of the virus. There is also included information on foot-and-mouth disease vaccine preparation and the tests applied in order to assess the immunizing value of vaccines, including examination of the antibody levels of vaccinated cattle, for which purpose the use of susceptible animals is emphasized.

Much research work has been and is being carried out on foot-and-mouth disease. A few examples will illustrate the position.

After observations with viruses of other animal diseases it was shown that phenolic extracts of animal tissues infected with foot-and-mouth disease, although containing no virus, can set up symptoms indistinguishable from typical foot-and-mouth disease.

It has been shown that some sites in susceptible animals are more sensitive in detecting virus than others.

It has been possible, by differential ultracentrifugation, to separate two fractions of the virus, one larger than the other. The larger fraction, containing roughly spherical particles in clusters or hexagonal arrays which can be seen under the electron microscope, is infective and fixes complement. The smaller fraction is non-infective but also fixes complement. If the virus is allowed to diffuse towards homotypic serum in agar gel, two precipitation lines are produced. It appears that two antibodies exist and that the larger component of the virus combines only with the homotypic serum, whereas the smaller component combines with the heterotypic serum. Using the agar gel precipitation technique it is possible to recognize sub-types within the types of foot-and-mouth disease virus.

Reference is made to the affinity of the virus for muscle, causing degeneration

in unweaned mice, young guinea pigs and calves. These observations may explain, to some extent, the better immunity following the injection of cattle intramuscularly than by other routes, with virus strains so attenuated that myotropic properties are enhanced and epitheliotropic properties are diminished. It has also been shown that severe degeneration of the pancreas occurs in infected guinea pigs with a high concentration of the virus in that tissue.

These are examples of some of the interesting research work in progress, much of which is fundamental in character, with the results bearing on viruses in general as well as leading to a better appreciation of the nature and action of the foot-and-mouth disease virus.

METABOLIC DISEASES OF RUMINANTS

This article is largely a summary of present information, concerning which 44 references are given. The main metabolic diseases are classified as those characterized by disturbance in the mineral composition of the blood – milk fever and grass tetany (also called lactation tetany, grass staggers or hypomagnesaemic tetany); those characterized by an abnormal accumulation of metabolic products or ketones in the blood, often accompanied by a reduction in the concentration of blood sugar – acetonaemia (ketosis) in dairy cattle and twin-lamb disease or pregnancy toxaemia of ewes; and those characterized by acute indigestion, accompanied by gross distention of the rumen by gas – "bloat".

Milk Fever

Early work is referred to concerning the decrease in the concentrations of calcium and inorganic phosphorus in the blood plasma in affected cows and the response to injections of suitable preparations of calcium; increased susceptibility to the condition with age and its greater prevalence in high-producers of milk and in some other animals are also discussed. The normal occurrence at parturition is depression in concentrations of calcium and inorganic phosphorus and a slight rise in magnesium in the blood, the whole position reverting to normal in three to ten days after parturition. These depressions increase with the age of the cow. From these findings it is concluded that "milk fever is the result of an exaggeration of changes which normally occur at parturition and the onset of lactation". Although this depression in calcium in the blood occurs it does not fall to a dangerous level at normal parturition and the extra calcium required for the milk is drawn from the food and the bones. Work on sheep has shown that during pregnancy and lactation some calcium and phosphorus are withdrawn from the bones even when there is abundance of these elements in the food. It is stated that this probably also happens in cows and "the fact that the incidence of milk fever increases with age of the animal is compatible with the concept which is generally believed that bone salts are less readily mobilized with increasing age".

The hormone of the parathyroid gland is concerned with mobilization of calcium and phosphorus from bones and promotes excretion of phosphate by

the kidney; there is also some evidence that it increases absorption of calcium from the gut.

Reference is made to "steaming-up" as a preventive of milk fever, i.e. increased feeding during the last two months of pregnancy and the daily administration of 10–30 million units of vitamin D for three to eight days before calving to increase absorption of calcium from the gut.

Grass Tetany

In this condition, which occurs in both lactating cattle and sheep, there is always a marked decrease in the concentration of magnesium in the blood plasma and a decrease in the calcium level in 75 per cent or more of the affected animals. There is a seasonal fluctuation in the concentration of magnesium in the blood of cattle kept out-of-doors all the year round, a decrease being more marked in winter and in severe weather. There is also a decrease in the blood of housed cattle when they are turned out to graze in the spring, the amount varying in individual animals. Restricted feeding may also influence the magnesium blood level.

Work on rats has shown the effect on their health of feeding rations low in magnesium and the effect of the level of magnesium in the blood. With very low levels of magnesium, nervous symptoms occurred, followed by convulsive seizures and death; the bones lose about one-third of their magnesium. Magnesium deficiency in calves resembles the conditions produced in rats, although calves seem to need more magnesium in relation to their size.

The concentration of magnesium in blood plasma reflects closely the quantity of magnesium available to the animal: in many cows the concentration decreased when they go out to grass in the early summer. Evidence from experiments with stall-fed cattle indicates that the decrease in urinary excretion of magnesium when grass is fed varies considerably from animal to animal. It seems that a reduction in intake of magnesium does not seem large enough to account for the changes in the blood and urine unless the magnesium in the grass is not so easily absorbed as magnesium in fodder or other winter foodstuffs.

Manurial treatment of pastures is considered. The more outstanding points concern observations in Holland, confirmed in Ireland, that the disease is common on pastures which have received heavy dressings of nitrogenous and potash manures and that the incidence increases with the amount of potassium applied. It was found in England that heavy nitrogenous dressings alone will increase the incidence. In experiments in which plots were dressed with potassium there was some small decrease in magnesium and calcium and it is stated that "as recovery of the temporary decrease in the concentration of plasma magnesium occurs without any increase in the magnesium content of the herbage it seems as if some other factor must also be concerned in producing the low concentration of magnesium in the blood". An interesting observation is that the occurrence of the convulsive syndrome does not seem to be closely related to the concentration of magnesium in the plasma. It is finally stated that "whatever is the final cause that precipitates seizures, there seems to be no doubt that the application of magnesium dressings to pastures or the

incorporation of adequate amounts of magnesium in concentrates gives good protection against the condition occurring, so that a practical means of prevention is available".

Acetonaemia in Cattle (Ketosis)

This condition, in which an abnormal amount of ketone bodies is found in the blood, is stated to occur either as a complication of other conditions or simply as a result of starvation. The mild disease may occur a day or two after calving but the more marked form is found up to a few weeks after parturition, mostly at the peak of lactation. In the clinical disease there is a depression of circulating glucose as well as an increase in ketone bodies. It is stated that "ketosis in a biological sense means that the products of metabolism of body tissues or of absorbed nutrients are not being oxidized rapidly enough to prevent the synthesis of excessive quantities of ketone bodies". Most ketone bodies are derived from fatty acids produced in the metabolism of body fat or from the digestion and absorption of fats in the food; certain fatty acids derived from proteins also make an important contribution. There are two main theories on the causation of excessive ketone bodies: (1) "the oxidative pathway becomes inadequate", which implies that the addition of carbohydrate derivatives will cure the condition; or (2) it is due to "excessive formation of acetyl coenzyme A", probably from disturbance of the endocrine control.

It is reported that success has followed the use of cortisone and hydrocortisone acetate in severely affected cattle.

It is finally stated that "it is not possible to offer any complete explanation of the origin of ketosis. It may be that the mild types of acetonaemia can be prevented or controlled by careful attention to the food and to the comfort of the animal".

Reports from the U.S.A. indicate that repeated large doses of sodium propionate have successfully treated acetonaemia.

Pregnancy Toxaemia in Sheep

This condition is essentially the same as ketosis in cattle but is found only in pregnant ewes, especially those carrying more than one lamb. It occurs when the lambs are growing rapidly *in utero*, towards the end of pregnancy, and it can be precipitated by suddenly starving the animals. Fat ewes are more likely to suffer than lean animals. The disease is found when there is a rapid change from high to low-level nutrition. The endocrine system is certainly involved but it is not yet understood what takes place. It has been shown recently, in large-scale field trials, that keeping ewes lean during the early stages of pregnancy and providing supplementary feed during the latter stages reduces the occurrence of the disease to negligible proportions. Rapid recovery takes place after successful parturition.

Bloat

In the article it is stated that in bloat in animals feeding on leguminous plants and purely grass leys the excessive and rapid foam formation cannot be explained. The sudden onset seems to have no relationship to the composition

of the food, although plant proteins may contribute to the foaming which occurs in the rumen of cattle on lush pastures. The gas formation occurs as a result of rapid fermentation, especially of soluble sugars in young green grass and from the liberation of carbon dioxide from the bicarbonate which enters the rumen in the saliva, as well as from the blood in exchange for the organic acids produced as a result of fermentation in the rumen and directly absorbed from it.

Antibodies were first used in the U.S.A. in the control of bloat. It was found that penicillin prevents foaming by partially suppressing fermentation. It has, however, to be given to each animal every third day during the bloating season. In time it causes resistance in some micro-organisms in the rumen. Affected animals may be treated by the administration of vegetable or mineral oils which effect rapid cures if given before the acute state is reached. Spraying of pastures with emulsions of vegetable oils has been shown to be an effective prevention in New Zealand; it is, however, applicable only where strip-grazing is practised. Attention is being given in New Zealand to the possibility that natural anti-foaming agents, e.g. plant fats and proteins, may be destroyed in the rumen, followed by excessive foaming. The main cause may be variations in the bacteriological population of the rumen, the reasons for which are not yet known. From studies with identical twins there are indications that bloat is concerned with some inherited factor.

BLOOD TYPES IN CATTLE

The study of blood groups in cattle was much influenced by observations on blood grouping in human beings. The simple technique which had been effective with human blood gave disappointing results with cattle blood. It was found that the antibodies which detected differences of compatibility were not present when cattle blood was subjected to tests but were built up later after repeated injections of red blood cells. Blood grouping has now become the most promising tool for the recognition of genetic factors, acting on the simple interpretation that one gene determines one antigenic blood character which is detected by the corresponding type of antibody. It is stated that "this method of recognizing the effect of a single gene will permit definite statements to be made about the genetics of individual animals". Many distinct blood characters are known in cattle, most of them being detected by reactions with antibodies. Further, variant forms of other blood constituents e.g. haemoglobins, are being disclosed and are expected also to be of much importance in detailed genetic analysis of cattle. "The future occurrence of each distinct type of antigen or haemoglobin is fixed at the moment of conception according to the laws of Mendelian genetics, and this is also true of certain serum proteins which are under investigation today".

Antigens are described as substances in red blood cells which combine with antibodies or stimulate the production of antibodies after injection. They are integral parts of the red blood cell and those which have been fully examined have been shown to be polysaccharide in nature. There are more than 30 antigens now widely known.

Antibodies are composed of normal proteins of the blood and their molecules have become specially adapted to the corresponding antigen. They can be elaborated only within the animal and most of them can be effectively stored in the frozen state for years. They are produced by repeated injection of blood from one ox into another and rabbits, sheep and goats may also be used. The injected animal does not produce antibody to those antigens in the injected blood which the recipient animal itself possesses.

"Cross-reactions" occur sometimes, because of lack of specificity, in antigen-antibody reactions; some preparations of antibody will react with antigens which have a slightly different genetic control.

Two types of haemoglobin are described – "fast" and "slow", distinguished by the different rates at which the molecules migrate under the influence of an electric current. Evidence is quoted that, in Britain, only Channel Island and South Devon cattle possess both types. Some confusion may occur with foetal haemoglobin normally present in calves.

During the last three years it has been found that there are several types of bovine serum. This has been shown by methods of analysis similar to those used in haemoglobin work and further investigations are in progress.

Each type of antigen, haemoglobin and serum protein has its own genetic control, and the influence of dozens of distinct genetic factors, or genes, can be traced in a few drops of cattle blood. The problem now is to trace how these genetic factors are transmitted from generation to generation. Some are inherited independently and others in groups of "systems", each system having two or more genetic factors, up to 20. Each animal receives only two genes belonging to any one system (one gene from each parent).

Information is given in the article on the blood type systems in cattle.

One section deals with the application of blood typing. The following are brief descriptions. Association with production: it has been shown that certain types of poultry are more productive than others and that it is financially advantageous to produce from them. There is also evidence from statistical analysis that one blood type is associated with high milk yield in cattle and another with depressed yield. Association with disease: it has been shown in human beings that those with particular blood groups are more likely to contract certain diseases. Haemolytic disease in the newborn: it has been found in the human subject, the horse and the pig that this condition occurs because of damage to the red blood cells of the offspring by the antibodies of the mother; it is suspected in cattle. Linkage with other factors: cattle may carry deleterious or unwanted genetic factors which are recessive and not distinguishable, unless two such genes are inherited by the same animal, e.g. close linkage with the gene for horus, when it would be possible by identifying the blood type to determine the sire of calves. Identification of inbreeding: it has been found that in Shorthorn cattle, where breeding was once concentrated in a few animals, there is a reduced variety of blood types. Twins: blood typing is being increasingly applied to the identification of one-egg and two-egg twins and freemartins. Blood transfusion: it is thought that more use would be made of blood transfusion in treating diseases

of cattle if safety can be assured. Clarification of doubtful parentage: in this connection there is need for internationally acceptable blood type certificates which, in turn, concerns international agreements on standardized reagents and notation.

Looking into the future, it is stated that the problems of immediate practical significance are expansion of the materials and techniques which now exist in Britain for the provision of a blood typing service (such a service already exists in some other countries) and work on the safety of blood transfusion in cattle. In the more distant future, it is considered that work should be undertaken on resistance to infections and on the health and economic value of animals from a knowledge of the parents' blood types.

BOOK REVIEWS

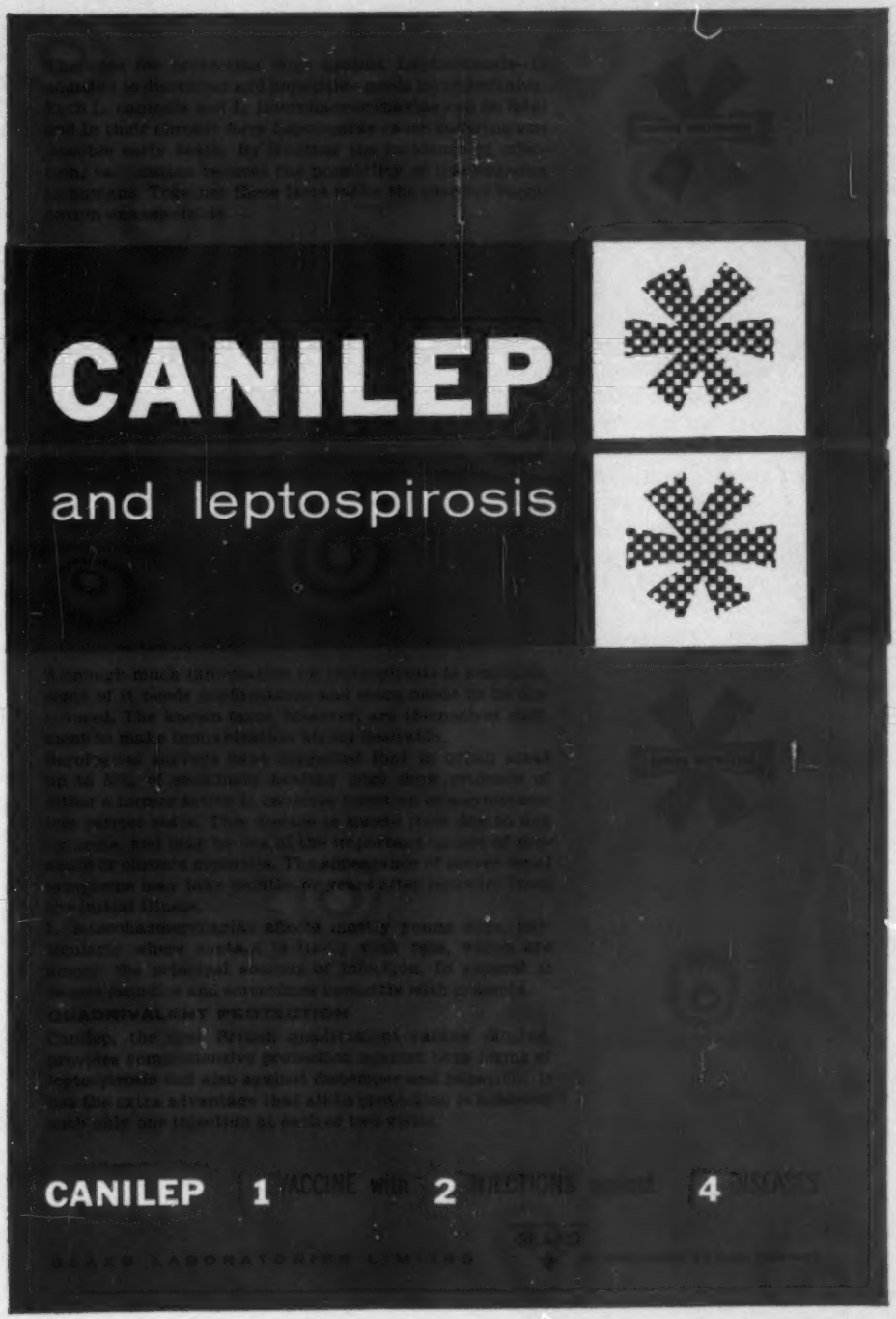
Symposia of the Institute of Biology, No. 9: Biological Problems arising from the Control of Pests and Diseases, edited by R. K. S. Wood. 25s.

This symposium deals with the side effects associated with modern methods used for the control of pests and diseases of man, his domestic animals and crops. It is particularly appropriate and welcome and serves to emphasize that although control measures are necessary economically for many modern-day problems, more widespread effects may occur which at first may not be apparent. This is illustrated by the recent reports of poisoning of foxes by Dieldrin seed dressings and other reports from the U.S.A. of D.D.T. poisoning in wild birds.

The symposium covers a wide selection of subjects, including the biological problems associated with chemicals for pest control, drug-resistant bacteria, the social consequences of malaria control and mental health. Since almost any method of control will have some undesirable side effects it is necessary to assess the most efficient method of using the control procedure to produce the greatest efficiency in control and to minimize the side effects. Contained within the pages of this small volume, which is very readable and well produced, are an abundance of examples of the biological effects of various control measures. For example the elimination of the screw-worm from the southern States of the United States of America is likely to increase the wild deer population; the aerial spraying of D.D.T., which is usually non-toxic for mammals, may be highly toxic for fish and cause severe mortality in insectivorous birds, and there may be a resurgence of plant pests due to the accidental destruction of their natural enemies by various insecticides.

The biological problems arising from the control of diseases in animals is adequately dealt with and the danger of animal over-population, especially where religious and social customs prevent their slaughter, is discussed and it is mentioned that this leads to a deterioration of animals and eventually in many areas to soil erosion. Further problems are associated with the use of live viruses for vaccination procedures and the consequent inability to eradicate disease from countries where the disease is endemic. It is stressed that the education of the population of certain countries is a very important aspect of disease control. The biological effect of the control of human disease in tropical areas is admirably dealt with and includes a consideration of the loss of immunity when endemic disease is controlled and the consequent increase of susceptibility in populations, particularly populations adjacent to areas where disease is still endemic. Further consideration is given to the effect of the elimination of diseases which stagnate economy and result in a population with a morale which is moribund; this leads to a reduced death rate with no reduction in fertility and, in the absence of adequate birth control and education, to a decline in the standard of living and essentially a return to conditions as they previously were.

Other chapters deal with the control in Africa of the tsetse fly, which is popularly quoted as being a preserver of African soil from destruction by man.


CANILEP
and leptospirosis
CANILEP 1 2 4

BOOK REVIEWS

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The case for protecting dogs against Leptospirosis—in addition to distemper and hepatitis—needs no underlining. Both L. canicola and L. icterohaemorrhagiae can be fatal and in their chronic form Leptospirae cause suffering and possible early death. By limiting the incidence of infection, vaccination reduces the possibility of transmission to humans. Together these facts make the case for vaccination unanswerable.

CANILEP

and leptospirosis

Although much information on leptospirosis is available, some of it needs confirmation and more needs to be discovered. The known facts, however, are themselves sufficient to make immunisation highly desirable.

Serological surveys have suggested that in urban areas up to 50% of seemingly healthy dogs show evidence of either a former active L. canicola infection or a symptomless carrier state. This disease is spread from dog to dog by urine, and may be one of the important causes of subacute or chronic nephritis. The appearance of severe renal symptoms may take months or years after recovery from the initial illness.

L. icterohaemorrhagiae affects mostly young dogs, particularly where contact is likely with rats, which are among the principal sources of infection. In general it causes jaundice and sometimes nephritis with uraemia.

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The preservation of African game is considered and it is pointed out that when the tsetse fly is controlled and land is properly farmed the large game cannot live in such circumstances.

This is a fascinating volume and should be read by all who are interested in disease control at the international level. It should also serve as a constant reminder to all in research that the solution of one problem may lead to the recognition of many other problems and certainly when this involves the human population the effects may be quite serious.

E. J. L. SOULSBY.

Virus Meningo-Encephalitis. Ciba Foundation Study Group No. 7. Editors: G. E. W. WOLSTENHOLME AND MARGARET P. CAMERON. London: Churchill. Price 12s. 6d.

This booklet is a report of a Ciba Foundation meeting (in honour of Professor Todorović, Belgrade), in which neurologists, physicians interested in infectious diseases, and neuropathologists participated in a discussion on the important problem of human viral meningo-encephalitis. Except in a limited way, it contains little of any great value to veterinarians other than those actively engaged in studies in virology and neuropathology. This does not detract from the value of the report. The viruses concerned in this particular neurological problem were restricted to the Coxsackie groups, the ECHO (enteric cytopathic human orphan) group, and the arbor (arthropod-borne) group, and it is only in the last-mentioned group there is any veterinary implication (e.g. louping-ill). It may be astonishing to many not in virological work to find out how new techniques in isolation of viruses, in this past ten years or so, have revealed an almost bewildering array of new viruses. As this has been accomplished so an equal variety of new neurological problems have confronted medical workers.

A large number of viruses in the above groups may all cause meningo-encephalitis in human beings and there are no clearly specific clinical signs and symptoms which have proved to be diagnostic, and virus isolation is essential for diagnosis. Many years ago, no doubt all such cases were regarded as "poliomyelitis" or aseptic meningitis. Of particular interest to veterinary pathologists might be the articles on the pathology of Coxsackie and Coxsackie-like virus infection (by W. F. Scherer), the tick-borne encephalitis complex, which includes louping-ill (by D. Slonim), the significance of inclusion bodies in viral meningo-encephalitis (by J. Bethlem) and a very interesting discussion by a clinical neurologist (A. M. G. Campbell) on post-infectious encephalitis in man—incidentally a condition which has still to be found in domestic animals. The inclusion body problem related to diagnosis is of equal importance to veterinary pathologists faced, as their medical confrères frequently are, with first finding bodies within cells, and then having to decide on their diagnostic importance. Bethlem relates that there are two kinds of inclusion bodies: (a) those consisting of a colony of elementary bodies surrounded by a matrix, and (b) those which are a product of reaction of cellular protoplasm to virus infection. In the former group are the inclusion bodies found in ectromelia (mouse pox), vaccinia and influenza, and in the latter the classical Negri body.

Intranuclear or cytoplasmic inclusion bodies do not necessarily betoken viral infection; the pathologist may think and suggest this but only the virologist can prove it by virus isolation. Some inclusions, such as those recognized in heavy metal poisoning, are not associated with any virus; in other conditions inclusion bodies have long been recognized, e.g. inclusion body, or subacute sclerosing, encephalitis in man, but all efforts to isolate a virus have failed. Further, the veterinary pathologist knows examples in animals where viral encephalitis is not associated with specific inclusion bodies. The chapter by Dr. Campbell raises many clinical and pathological issues on the so-called post-infectious encephalitides in man, and also debates whether there is any relationship between "viral diseases" of cats and the occurrence of meningo-encephalitis in man without any clear decision being possible. The discussions which followed the various papers are lively, and contain items equally applicable to viral problems in animals.

J. R. M. INNES.

The Grass Crop—its development, use and maintenance. By WILLIAM DAVIES. Revised 2nd edition. London: Spon Ltd. Price 45s.

Anyone remotely connected with agriculture will welcome the second revised edition of *The Grass Crop*. Since 1952, progress in the fields of research and technology in matters pertaining to the use of grass in this country has necessitated the addition of new chapters to and considerable revision of the first edition. This may be regarded as a measure of the author's contribution to the acceleration of interest in what is undoubtedly our major crop.

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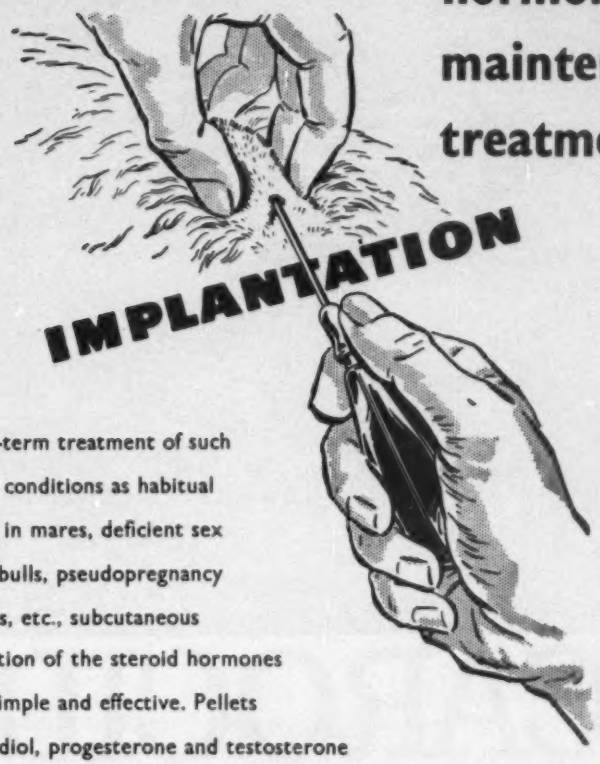
Dr. Davies describes his approach as a "kind of trinity study; the trinity of the sward, of the animal which browses upon it and of the soil which not only anchors but nourishes that sward". This balanced approach and the clarity of its theme give this book that rare quality of being informative and stimulating to a very wide circle of readers.

H. L. WILLIAMS.

The Female of the Species. By R. H. SMYTHE. London: Country Life Ltd. Price 21s.

R. H. Smythe has established himself as an accomplished contributor to the literature in Veterinary Science. He has also written a number of interesting books in the wider field of animal behaviour. It is into this latter category that the text now under review falls. The title of the book and the table of contents

hormone maintenance treatment



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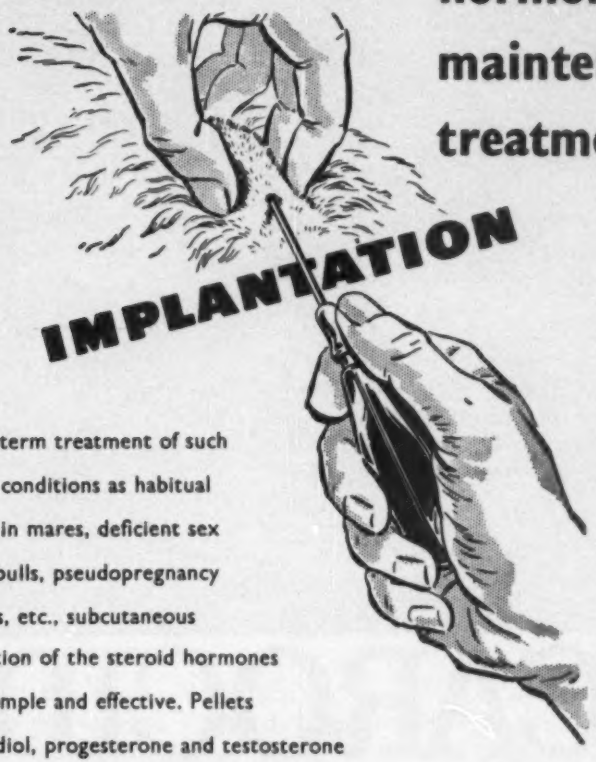

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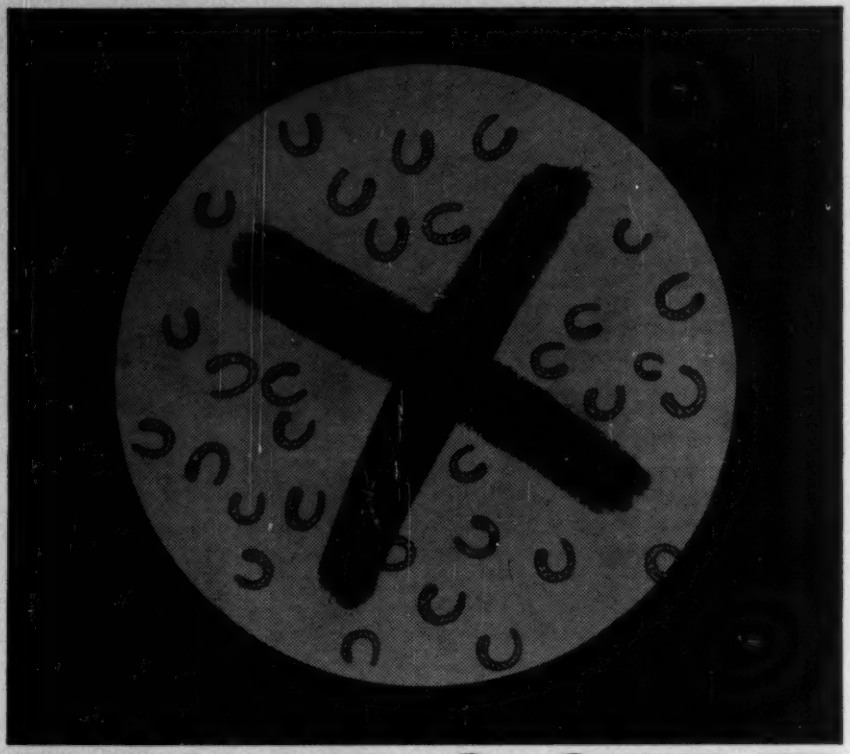

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are perhaps a little misleading, but the author in the early pages of the first chapter seeks to disperse any misconceptions by the assurance that he proposes to discuss the role of the female unhampered by the problems thrown up by civilization. The purpose of the book is to describe and explain the pattern of behaviour of the female as it is laid down for her throughout her reproductive life and to look at the differences as they occur in the different species of vertebrate life. To do this the author has drawn on his own observations in the field and on the experience and knowledge of a number of authorities. The book holds the interest and will probably act as a stimulus for a number of its readers to further study of the fascinating subject of animal behaviour.

C. S. GRUNSELL.

Pig Industry Development Authority. Third Annual Report. London: H.M.S.O. Price 1s.

The 43 pages of the report give an account of the work of the Authority for the year ended 30th September, 1960. It is written in general terms and includes sections on livestock improvement, research and development, bacon marking, pig grading, market research and finance and organization. The income and expenditure account shows a reduction in the surplus of income over expenditure of £265,870 as compared with the previous year. This was due to some extent to a fall in the Authority's levy income owing to the marketing of fewer pigs, and partly also to the fact that progeny testing is now being financed. The third and major cause for the reduction in the surplus was the development of the work of the Authority which, in the year covered by the Report, had an expenditure of £34,322 in support of research into the practical problems of the industry.

C. S. GRUNSELL.

Infectious Diseases of Domestic Animals. By W. A. HAGAN AND D. W. BRUNER. London: Baillière, Tindall & Cox Ltd. Price 90s.

This book must be well known to most veterinarians. It was originally published by the senior author as "an outgrowth of a lecture course on pathogenic bacteriology and immunology" given by him to students of veterinary medicine. Professor Hagan has since been joined by Professor Bruner and the fact that a fourth edition of this manual has been called for speaks for its value.

The authors make it quite clear that this is not a treatise on bacteriology, but rather a discussion of the infectious diseases of animals with special reference to their aetiological factors.

The authors classify the leptospiral infections according to their serological reactions. This may arouse some criticisms, but this chapter gives a very useful account of leptospiral infection in animals.

The book is a very useful one for consultation and within the scope of some 1,000 pages covers adequately the infectious diseases of animals.

It is well produced and the illustrations are good.

The book can be recommended.

H. G. LAMONT.

Crop Husbandry. By R. D. Park. Oxford University Press. Price 18s.

This well-written book deals admirably with the many and varied facets of crop husbandry. It explains concisely the reasons for a number of farming operations. Subjects such as soil types, land drainage, fertilizers, grassland, weed control, etc., are presented in a most interesting and straightforward manner. The information provided should be of the greatest value to those who are interested in agriculture but have little theoretical knowledge or practical experience of farming.

The book is well illustrated with photographs and drawings. It is true that a few of the latter are not just as clear as one would wish.

Although intended primarily for students in farm institutes and young people attending part-time courses in agriculture, it could be read with advantage by veterinary students in connection with the animal husbandry course. This publication will no doubt also find its way on to the bookshelves of a number of practising veterinary surgeons working with farm livestock.

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The Hannah Dairy Research Institute, Kirkhill, Ayr.
— Potassium Chlorate as a Prophylactic Treatment for Subclinical Ketosis.

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